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Efficient Synthesis of α-C-Galactosyl Ceramide Immunostimulants: Use of Ethylene-Promoted Olefin Cross-Metathesis

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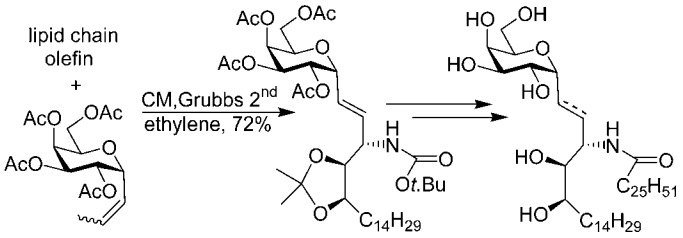
ABSTRACT



Olefin cross-metathesis has been used to prepare α-C-galactosylceramide derivatives. The metathesis process merged vinyl and propenyl glycosides with vinyl derivatives of phytosphingosine. The use of ethylene enhanced the yield of the methathesis step.

Agelasphins (α-galactosylceramides), a series of glycosphingolipids isolated from a marine sponge of the genera *Agelas*, showed potent effects on the immune system of mice.[1] This discovery led to the synthesis of a slightly simplified analogue KRN7000 (**1**) as the lead compound for further development.[2] Detailed studies revealed that **1** is a powerful immunostimulant which induces formation of both interferon-γ (IFN-γ) and interleukins (IL)-12 and (IL)-4 by first binding to antigen-presenting CD1d cells whereupon the resulting complex then binds to natural killer T (NKT) cells.[3] This group of cytokines, which induce antagonistic biological effects, i.e., Th1- and Th2-type responses, apparently limit α-GalCer from eliciting a maximum of either response.[4] Interestingly, a recent report describes aza analogues of **1** with good activity which apparently do not interact via NKT cells.[5]

Our recent report of C-analogue **2** showed that it was 1000 times more active than **1** in a mouse malaria assay and 100-fold more potent in a mouse melanoma model.[6] Our first synthesis employed the Ramberg−Backlund reaction as a key step for linking a galactose derivative to a homophytosphingosine which itself required a total synthesis.[7] To make

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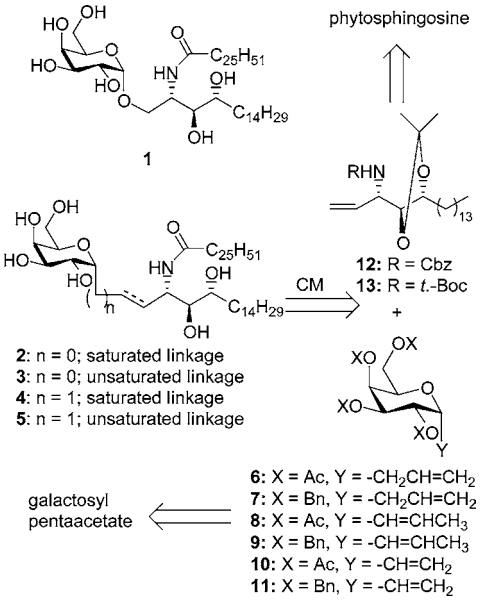
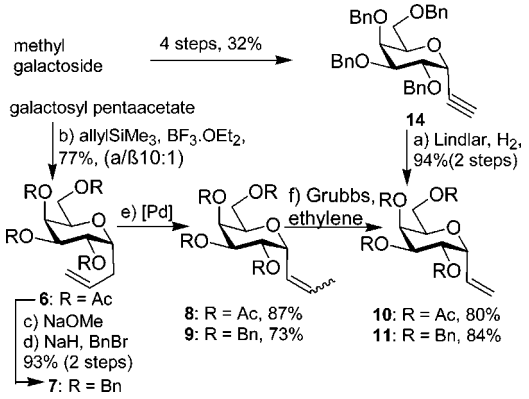


Figure 1. Cross-Metathesis Approach to C-Galactosyl Ceramide Analogues.

larger quantities of our potent analogue available to the immunology community, we have designed a shorter synthesis which is based on olefin cross-metathesis.[8] Here, we report our second-generation convergent synthesis, with a novel ethylene-promoted CM sequence as a key feature, of the exact C-galactosyl ceramide analogue **2**, its homologue with one added methylene **4**, and their unsaturated counterparts **3** and **5** (Figure 1).

Our convergent approach begins with preparations of α-C-vinyl and propenyl galactosides. The α-C-vinyl galactoside **11** was initially produced by controlled hydrogenation of the correspondent ethynyl sugars **14** (Scheme 1). The latter was made by using the protocol of Dondoni and Isobe.[9] Although this method worked smoothly in our hands, the overall yield (30% after five steps from methyl galactoside) was limited by the key C-glycosidation step. Thus, we investigated the feasibility of a preparation of **11** via transformation of the C-(1-propenyl) galactosides **9** to terminal olefins utilizing cross metathesis with ethylene. Key material **9** was readily produced from galactosyl pentaacetate using standard allyl C-glycosidation,[10] followed by palladium chloride mediated isomerization.[11] Subsequent treatment with Grubbs catalyst

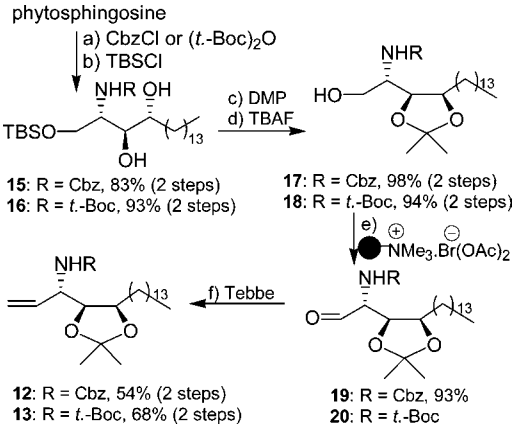
Scheme 1. Preparation of Sugar Olefins as CM Partners



(second generation) under an ethylene atmosphere afforded C-vinyl galactoside **11** in an overall 50% yield after five steps (Scheme 1). The α-C-vinyl galactoside with acetyl protection **10** was also prepared in good yield. This CM approach to C-vinyl glycosides has advantages over other available methods[8e−g,12] including the ethynyl sugar route because of the simpler chemistry involved.

The terminal olefin form of the sphingosine side chain was prepared starting from the commercially available phytosphingosine (Scheme 2). In the benzyl carbamate

Scheme 2. Preparation of Terminal Olefins of Phytosphingosine



sequence, after formation of the carbamate, the selectively blocked primary alcohol **17** was obtained in four protecting group manipulations in excellent yield. To obtain diol **15**, the primary alcohol was temporarily blocked by a silyl group, which was then removed after the clean isopropylidenation of the vicinal diol. Further transformation to the aldehyde

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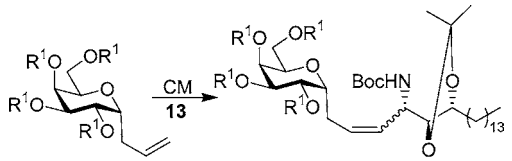
19 was not totally problem-free. Standard Swern oxidation[13] gave rise to epimerization at the α position, while a protocol with sodium hypochlorite catalyzed by TEMPO[14] led to the acid as a product of overoxidation. Finally, we resorted to the Kirschning solid phase oxidant in dilute neutral solution, which successfully effected the transformation to **19** in 93% yield.[15] Terminal olefins **12** and **13** (*t*-Boc) were obtained after treating the aldehydes with Tebbe reagent with overall yields of 46% and 60%, respectively.

The convergent olefin metathetic assembly was investigated with the above suitably protected coupling partners. C-Allyl glycosides (Table 1) participated well using Grubbs' catalyst (second generation) in coupling with the *tert*-butyl carbamate version of the lipid side chain in more than 60% isolated yield under nonoptimized conditions. Considering the bulky neighboring groups of both partner alkenes, we believe this yield to be good. There was no significant difference in reactivity between peracetyl- and perbenzyl-protected sugar olefins **6** and **7**. Vinyl homologue **11** (Table 2), comparatively, afforded significantly reduced cross-coupling yields (entries 1 and 2), possibly attributed to deactivating chelation between metal center, the multifunctional groups around the rigid tetrahydropyran ring, and the increased congestion at reaction sites.[16] This result is quite consistent with the reports from several other groups.[8e,g,17] With substituted sugar olefins, either protected with acetyl or benzyl groups (**8** or **9**), only traces of expected product were detected in our initial tries (entries 3−5).[8g] After optimization with respect to degassing, catalyst loading, temperature, solvents, and concentration, the CM starting with peracetyl-protected propenyl sugar **8** afforded cross-coupled product **26** with an isolated yield of 27% (entry 6). Under the same conditions, there was no improvement with benzylated propenyl sugar **9** as starting CM partner (entry 5).[18] As a result of our success in using ethylene for the conversion of C-(1-propenyl)glycosides to C-vinyl counterparts along with reports of ethylene promotion of enyne cross-metathesis ("Mori conditions"),[19] we tested ethylene as a promoter for our cross-coupling process. In the event, refluxing the side-chain olefin **13** with excess C-(1-propenyl)-sugar **8** (2.5 equiv) in the presence of ethylene with a cumulative addition of 10 mol % of second-generation Grubbs' catalyst in two portions led to greatly improved formation of CM product **26** with more than 70% isolated yield (entry 7). For this outcome, it must be the case that CM of the product with ethylene is a slow step. The observed enhancement of our CM by ethylene is probably the result of improved ruthenocyclobutane formation of the phytosphingosine partner. Hoye has recently reported an intramolecular relay method to improve difficult RCM reactions.[20] This also presumably promotes formation of slow-to-form ruthenocyclobutane intermediates.

Completion of the synthesis of our target molecules **2**−**5** requires amidation and deprotection of CM products **26** and

Table 1. CM Formation of C-Glycolipids with C-Allylsugars as Coupling Partners[a]

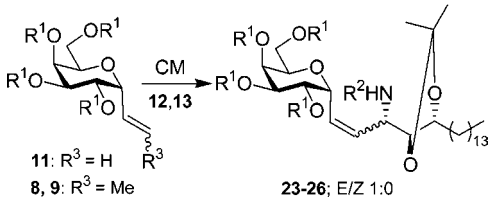


6: R^1 = Ac
7: R^1 = Bn
21: R^1 = Ac; E/Z 3:1
22: R^1 = Bn; E/Z 5:1

entry	sugar	solvent (*T* (° C))	product	yield[b] (%)
1	**6**	CH_2Cl_2 (reflux)	**21**	61 (54)
2	**7**	CH_2Cl_2 (reflux)	**22**	61 (48)

[a] Both reactions were allowed to stir for 2 d with excess sugar partners (2.5 equiv) and 30 mol % (in two portions) of Grubbs' catalyst (second generation). [b] All yields given are isolated ones, and those yields in parentheses refer to isolated homodimers of sugars.

Table 2. Effects of Protecting Groups, Olefin Substitutions, and Ethylene on CM Efficiency



11: R^3 = H
8, 9: R^3 = Me
23-26; E/Z 1:0

entry	sugar	lipid	solvent	product (R^1,R^2)	yield[a] (%)
1	**11**	**13**	PhH	**23** (Bn, Boc)	23 (10)
2	**11**	**12**	PhH	**24** (Bn, Cbz)	37 (9)
3	**9**	**13**	PhH	**23** (Bn, Boc)	trace
4	**8**	**12**	PhH	**25** (Ac, Cbz)	trace
5	**9**[b]	**13**	CH_2Cl_2	**23** (Bn, Boc)	trace
6	**8**[b]	**13**	CH_2Cl_2	**26** (Ac, Boc)	27 (56)
7	**8**[c]	**13**	CH_2Cl_2	**26** (Ac, Boc)	72 (46)

[a] All yields given are isolated ones, and those yields in parentheses refer to isolated sugar homodimers. [b] Optimized conditions: degassing the reaction mixture at the beginning; catalyst loading: 30 mol % in two portions with 24 h interval; 0.1 M in dry CH_2Cl_2; reflux. [c] Condition *b* with presence of ethylene and 10 mol % catalyst loading. All reactions were stirred under reflux for 2 d with sugar in excess (2.5 equiv).

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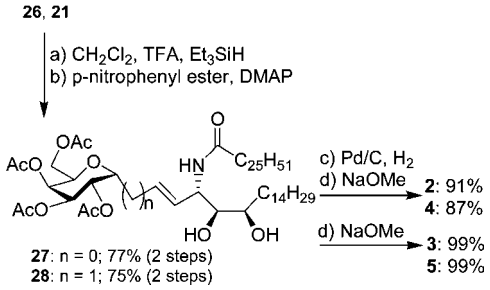
Scheme 3. Final Manipulation to Target Galactosylceramides



21 (Scheme 3). Only one step requires comment. Addition of triethylsilane in the acidic hydrolytic step[21] blocked a troublesome trifluoroacetylation.

We briefly describe three bioassays of alkene **3** with C-glycoside **2** and O-glycoside **1** as positive controls. In the mouse malaria assay, the animals were treated with glycolipid (or no treatment as control) and then challenged with an injection of sporozoites. After 48 h, the animals were sacrificed and their livers were assayed for sporozoites. Column 2 of Table 3 shows that both **2** and **3** are effective at the 1 μg level in blocking sporozoite viability. The results of two cytokine assays, IFNγ and IL-4, are shown in the remaining columns. In the IL-4 assay, the O-glycoside **1** has by far the most powerful effect. It is hypothesized that IL-4 and IFNγ are antagonistic.[4] Thus, the relatively low levels of IL-4 produced by the C-glycosides may permit a more effective stimulation of the NKT cell cascade as compared to the O-series. The time courses for IFNγ and IL-4 production are not correlated with each other or with the malaria results. Thus, one can tentatively conclude that variance of effects are not simply due to differences between O- and C-glycolipid lifetimes (which was the fundamental rationale for the study of C-analogues). Not shown is data for the C-glycoside homologues **4** and **5** where we have a

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Table 3. Three Bioassays of Galactosyl Ceramide Ligands

ligand	malaria[a] $\times 10^{-3}$	IFNγ[a] (pg/mL)	time (h) max IFNγ	IL-4[a] (pg/mL)	T (h) max IL-4	IFNγ/ IL-4
none	250	0		0		
1	25	1800	12	1100	2	1.64
2	<1	2100	24	475	2	4.42
3	<1	600	12	250	2	2.4

[a] 1 μg of ligand per mouse.

3-carbon (or 4-bond) linker. These materials are inactive. This is the first demonstration in α-galactosylceramides that a four-bond connector between galactose-C1 and ceramide C−N precludes their recognition by the receptors of the subject immune cascade.

In conclusion, we have developed a practical strategy for our second-generation synthesis of the α-C-galactosylceramide structure. This convergent construction exhibits very high efficiency with an overall yield of 30% after 11 steps for **2** (CRONY 101) starting from commercial phytosphingosine. Compared with our previous synthesis, it is more conveniently scaled up as a prelude for extensive bioassay research. Beyond our specific application, CM synthesis of C-vinyl glycosides and ethylene-promoted cross metathesis have potential application for the construction of glycoconjugates and also may lead to improvements in the general profile of cross metathesis.

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Supporting Information Available: Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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