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A concise synthesis of (3*S*,4*S*,5*R*)-1-(α-D-galactopyranosyl)-3-tetracosanoylamino-4,5-decanediol, a *C*-glycoside analogue of immunomodulating α-galactosylceramide OCH

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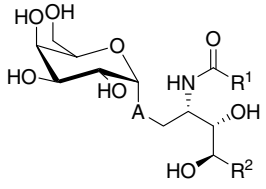
Abstract—A concise and convergent synthesis of the *C*-glycoside analogue **2b** of immunomodulating α-galactosylceramide OCH **1b** starting from readily available 2,3,4,6-tetra-*O*-benzyl-D-galactose **3** and L-arabinose **6** is described. The synthesis features the nucleophilic addition of an α-ethynyl sugar **5** to the phytosphingosine-precursor aldehyde **9** and would be applicable to a variety of *C*-glycoside analogues of interest.

Natural killer (NK) T cells are potent producers of immunoregulatory cytokines and specific for glycolipid antigens bound by a nonclassical major histocompatibility complex (MHC) class I-like molecule, CD1d.[1] The glycolipids, an α-galactosylceramide named KRN7000 **1a**[2] and an altered analogue termed OCH **1b** possessing a shorter C5 sphingosine side chain,[3] have been identified as NKT cell ligands (Fig. 1). Compound **1b** was shown to induce a predominant production of interleukin (IL)-4, a key Th2 cytokine engaged in autoimmunity control, over Th1 cytokine interferon (IFN)-γ, while **1a** induced both Th1/Th2 cytokines. Only compound **1b** but not **1a** is significantly effective in animal models of Th1-mediated autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) and collagen induced arthritis (CIA).[3,4] Quite recently, we have reported a practical and efficient synthesis of **1b** in 12 steps and 19% overall yield from commercially available D-arabitol.[5] It is often a conventional strategy to synthesize the *C*-glycoside analogue of biologically active *O*-glycosides, since *C*-glycosides are in general resistant to enzymatic degradation by glycosidases and may exhibit longer duration of action. It has recently been demonstrated that conversion of **1a** to its *C*-glycoside analogue **2a** leads to striking enhancement of activity on in vivo animal models of malaria and lung cancer



1a (KRN7000; A = O, R^1 = *n*-$C_{25}H_{51}$, R^2 = *n*-$C_{14}H_{29}$)
1b (OCH; A = O, R^1 = *n*-$C_{23}H_{47}$, R^2 = *n*-C_5H_{11})
2a (A = CH_2, R^1 = *n*-$C_{25}H_{51}$, R^2 = *n*-$C_{14}H_{29}$)
2b (A = CH_2, R^1 = *n*-$C_{23}H_{47}$, R^2 = *n*-C_5H_{11})

Figure 1. Structure of α-galactosylceramides **1a**,**b** and their *C*-glycoside analogues **2a**,**b**.

Keywords: *C*-glycoside; OCH; Ceramide; CD1d; NKT cell ligand.
Abbreviations: TMSOTf, trimethylsilyl trifluoromethanesulfonate; PTSA, *p*-toluenesufonic acid; MTPA, 2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid; DME, 1,2-dimethoxyethane; EDCI·HCl, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOAt, 1-hydroxy-7-azabenzotriazole.
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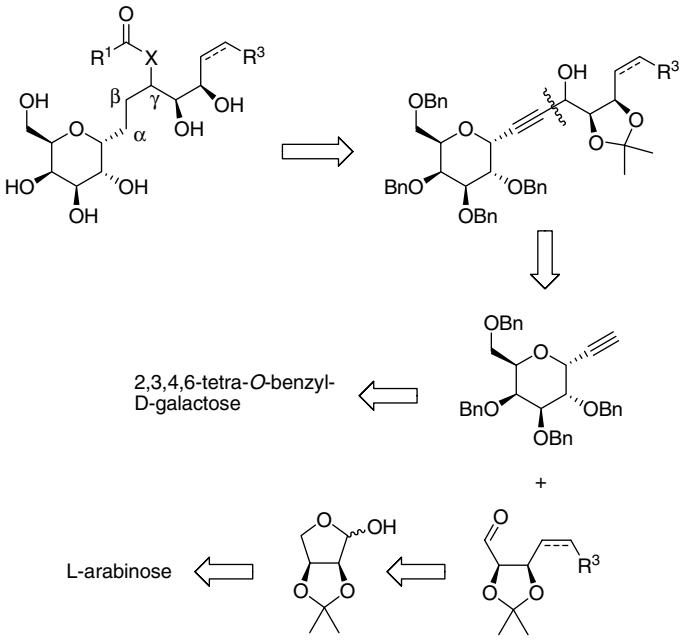


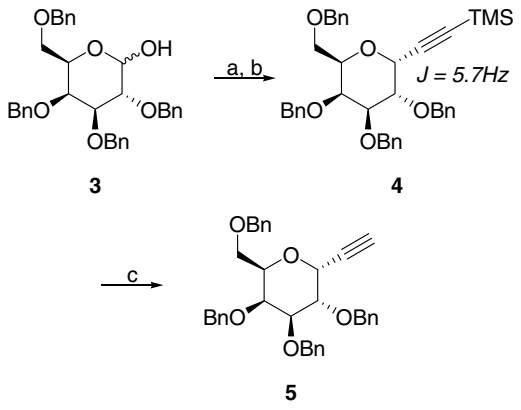
Figure 2. Retrosynthetic analysis of *C*-glycolipids.

by inducing prolonged production of the Th1 cytokines IFN-γ and IL-12.[6]

These results prompted us to investigate a short and versatile synthetic pathway applicable to the *C*-glycoside analogues of **1b** and related compounds. There have been only a few reports on the synthesis of **2a** to date, that utilize Wittig reaction,[7] Ramberg–Bäcklund reaction followed by β-selective hydrogenation from the diisopropylsilyl protecting group,[6b] or olefin cross-metathesis for the installation of the *C*-glycoside linkage.[8] We present herein a concise and short synthesis of **2b**, which is independent of previous methodologies for **2a** and would be applicable to a variety of phytosphingosine derivatives in terms of chain length and substitution, including aromatic groups and heteroatoms.

Our retrosynthetic analysis for **2b** revealed a straightforward and versatile strategy based on the nucleophilic addition of an α-ethynyl sugar to the phytosphingosine-precursor aldehyde synthesized from L-arabinose (Fig. 2). The critical feature of our strategy is the dissecting position, which former retrosyntheses of **2a** all cleaved between the α- and β-carbons of the anomeric center, while we chose between the β-and γ-carbons. This allowed the shorter and expansive synthesis at the expense of a need to construct a new stereocenter.

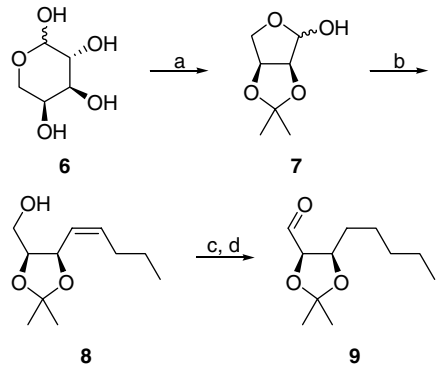
Perbenzylated 1-deoxy-1-α-ethynyl-galactopyranose **5** was prepared from commercially available 2,3,4,6-tetra-*O*-benzyl-D-galactose **3** in three steps (Scheme 1).[9] Thus, compound **3** was quantitatively acetylated and α-face selectively coupled with tri-*n*-butyl(trimethylsilylethynyl)tin in the presence of TMSOTf and MS-4A to give **4** ($^3J_{\text{anomericHH}}$ = 5.7 Hz) in 60% yield. The corresponding β-isomer of **4** was not detected but a small amount of **3**, presumably due to hydrolysis of the acetylated



Scheme 1. Reagents and conditions: (a) Ac_2O, pyridine, CH_2Cl_2, 0 °C to rt (quant.); (b) tri-*n*-butyl(trimethylsilylethynyl)tin, TMSOTf, MS4A, CH_2Cl_2, rt (60%); (c) 1 N NaOH, MeOH, CH_2Cl_2, rt (quant.).

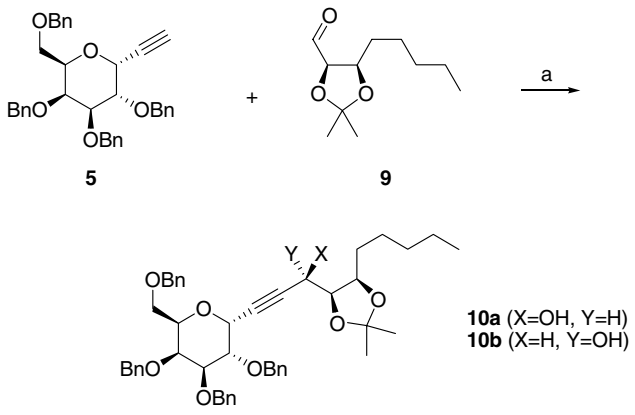
substrate, was detected by TLC. Desilylation of **4** was accomplished by NaOH–MeOH to give the desired compound **5** quantitatively.

The counterpart **9** was efficiently synthesized from L-arabinose **6** endowed with suitable stereochemistry corresponding to the vicinal hydroxyl groups in the phytosphingosine moiety (Scheme 2). Thus, compound **6** was protected as a 3,4-*O*-acetonide, reduced to a triol and subjected to $NaIO_4$ degradation to produce 2,3-*O*-isopropylidene-L-erythrose **7**[10] in 71% overall yield. Four carbon homologation by Wittig reaction followed by hydrogenation and standard Swern oxidation of the primary alcohol produced the requisite aldehyde **9** in 61% overall yield. No epimerization of **9** was observed neither during the reaction nor after storing overnight at −20 °C.



Scheme 2. Reagents and conditions: (a) (i) $Me_2C(OMe)_2$, PTSA, DMF, rt, (ii) $NaBH_4$, EtOH, rt, (iii) $NaIO_4$, H_2O, rt (71%; three steps); (b) *n*-$BuPh_3PBr$, *n*-BuLi, THF, −78 °C to rt (79%); (c) H_2, Pd/C, EtOH, rt (96%); (d) DMSO, $(COCl)_2$, CH_2Cl_2, −78 to 0 °C (80%).

Next, the coupling reaction of **5** and **9** was examined using various metal acetylide ions. This key step requires the generation of an adequate stereochemistry at the epimeric propargylic hydroxyl group. When the lithium acetylide derived from **5** was employed, the reaction gave a 3.2:2 mixture of diastereomers, **10a** and **10b**, in 47% and 30% yields, respectively, on the basis of the recovered starting materials[11] (Scheme 3). Compounds **10a** and **10b** were easily separated by column chromatography over silica gel using *n*-hexane/EtOAc (10:1). The stereochemistry of the propargylic hydroxyl group was determined for the more polar isomer **10a** to be the *R*-isomer by applying modified Mosher's protocol (Fig. 3).[12,13] Other attempts of chelation-controlled addition reaction utilizing Zn or Mg species[14] did not improve the diastereoselectivity and decreased the chemical yields.[15]



Scheme 3. Reagents and conditions: (a) *n*-BuLi, THF, −48 to −30 °C (47% yield for **10a**, 30% yield for **10b**).

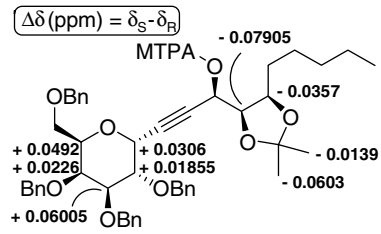
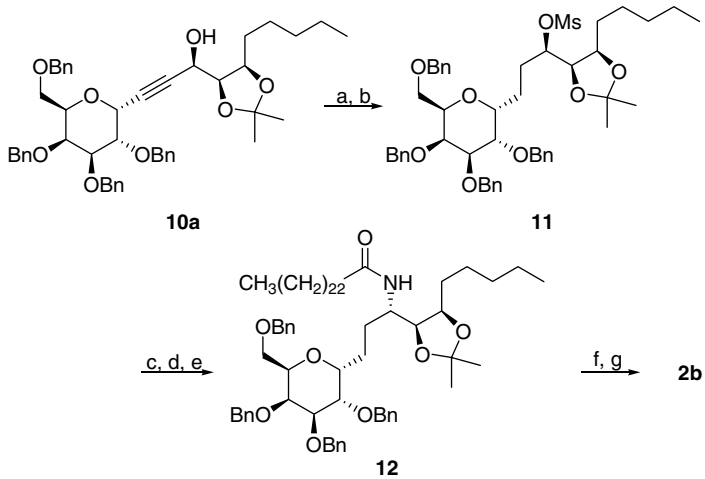


Figure 3. $\Delta\delta$ values for the MTPA esters of **10a**.

Once the key intermediate **10a** was available, the synthesis of **2b** was completed in a straightforward and efficient manner (Scheme 4). Thus, reduction of the triple bond in **10a** with $TsNHNH_2$ followed by mesylation of the hydroxyl group gave **11** in 86% yield. Compound **11** was azidated, reduced to an amine by hydrogenation and consecutively acylated with *n*-tetracosanoic acid to afford amide **12** in 48% overall yield. Finally, deprotection of the isopropylidene acetal of **12** under acidic conditions and subsequent removal of the benzyl groups by hydrogenation furnished **2b** in 88% yield. The synthetic sample displayed satisfactory ^1H NMR spectrum and was confirmed by high-resolution mass spectrum



Scheme 4. Reagents and conditions: (a) $TsNHNH_2$, DME, NaOAc aq, reflux (91%); (b) MsCl, Pyridine, CH_2Cl_2, 0 °C to rt (94%); (c) NaN_3, DMF, 90 °C; (d) H_2, $Pd/CaCO_3$, EtOH, rt; (e) tetracosanoic acid, EDCI·HCl, HOAt, Et_3N, DMF–CH_2Cl_2, rt (48%; three steps); (f) 80% AcOH, 60 °C (88%); (g) H_2, $Pd(OH)_2/C$, MeOH–CH_2Cl_2, rt (quant.).

(HR-MS).[16] Compound **2b** did not show in vitro IL-4 and IFN-γ production in splenocytes but increased serum level of IL-4 in C57BL/6 mice in vivo. Thus, compound **2b** proved to possess the pharmacological profiles distinctively different from that of **1b** from the preliminary results of biological testing.[17] Similar pharmacological differences have been reported between **1a** and its *C*-glycolipid **2a**,[6] which support our findings.

In conclusion, we have developed a concise protocol for the synthesis of **2b** involving only 12 steps starting from commercially available 2,3,4,6-tetra-*O*-benzyl-D-galactose **3** and L-arabinose **6**. Although the coupling reaction of **5** and **9** proceeded but not yet with sufficient stereoselectivity, the total sequence of this method is more convergent and versatile compared with those previously reported for **2a**,[6b,7,8] since the two obtained diastereomers **10a** and **10b** can be easily separated by conventional purification procedures. Consequently, this new synthetic route would enable the synthesis of a variety of *C*-glycoside analogues of phytosphingolipids related to **1a** and **1b**, especially those which vary in the sphingosine side chain length or substituents other than aliphatic alkyl groups. In addition, it should be noted that this route promises to contribute significantly for clarifying the structure–activity relationships (SARs) of this series of *C*-glycosides of interest. The SAR study in this series of compounds will be reported in due course.

Acknowledgements

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13. ^{1}H NMR of (*S*)-MTPA ester of **10a** (400 MHz, $CDCl_3$): δ 7.6–7.55 (m, 2H), 7.4–7.2 (m, 23H), 5.65 (dd, 1H, J = 7.8 Hz, 1.7 Hz), 4.90 (d, 1H, J = 11.4 Hz), 4.83 (dd, 1H, J = 5.8 Hz, 1.7 Hz), 4.77 (d, 1H, J = 11.7 Hz), 4.73 (d, 1H, J = 11.8 Hz), 4.69 (d, 1H, J = 11.7 Hz), 4.64 (d, 1H, J = 11.8 Hz), 4.54 (d, 1H, J = 11.4 Hz), 4.44 (d, 1H, J = 11.9 Hz), 4.37 (d, 1H, J = 11.9 Hz), 4.18 (dd, 1H, J = 7.6 Hz, 5.8 Hz), 4.08 (dd, 1H, J = 9.7 Hz, 5.7 Hz), 4.15–4.05 (m, 1H), 4.01 (t, 3H, J = 6.2 Hz), 3.95–3.93 (m, 1H), 3.78 (dd, 1H, J = 9.8 Hz, 2.7 Hz), 3.49 (s, 3H), 3.5–3.4 (m, 2H), 1.6–1.5 (m, 2H), 1.55–1.4 (m, 1H), 1.39 (s, 3H), 1.27 (s, 3H), 1.3–1.1 (m, 5H), 0.82 (t, 3H, J = 6.5 Hz); ^{1}H NMR of (*R*)-MTPA ester of **10a** (400 MHz, $CDCl_3$): δ 7.6–7.57 (m, 2H), 7.5–7.2 (m, 23H), 5.66 (dd, 1H, J = 8.9 Hz, 1.8 Hz), 4.90 (d, 1H, J = 11.4 Hz), 4.80 (dd, 1H, J = 6.0 Hz, 1.8 Hz), 4.77 (d, 1H, J = 11.7 Hz), 4.69 (d, 1H, J = 11.7 Hz), 4.68 (d, 1H, J = 11.7 Hz), 4.61 (d, 1H, J = 11.7 Hz), 4.55 (d, 1H, J = 11.4 Hz), 4.45 (d, 1H, J = 11.9 Hz), 4.37 (d, 1H, J = 11.9 Hz), 4.26 (dd, 1H, J = 8.9 Hz, 5.7 Hz), 4.11 (ddd, 1H, J = 13.6 Hz, 5.7 Hz, 3.2 Hz), 4.06 (dd, 1H, J = 9.8 Hz, 5.9 Hz), 3.96 (t, 3H, J = 6.4 Hz), 3.93–3.91 (m, 1H), 3.72 (dd, 1H, J = 9.8 Hz, 2.8 Hz), 3.57 (s, 3H), 3.5–3.4 (m, 2H), 1.75–1.55 (m, 2H), 1.55–1.5 (m, 1H), 1.40 (s, 3H), 1.32 (s, 3H), 1.3–1.1 (m, 5H), 0.81 (t, 3H, J = 6.8 Hz).
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15. The total chemical yield of **10a**,**b** was decreased to 39% with a similar diastereomeric ratio when the magnesium acetylide of **5** was used in this reaction. The zinc acetylide derived from **5** produced only a trace of **10a**,**b**.
16. Data for **2b**: $[\alpha]_D^{28}$ +9.3 (*c* 0.10, pyridine); ^{1}H NMR (400 MHz, pyridine-d_5): δ 8.45 (d, 1H J = 7.3 Hz), 5.2–5.05 (m, 1H), 4.74 (dd, 1H, J = 8.9 Hz, 5.4 Hz), 4.6–4.45 (m, 3H), 4.37 (dd, 1H, J = 11.2 Hz, 4.6 Hz), 4.25 (dd, 1H, J = 8.9 Hz, 3.4 Hz), 4.25–4.15 (m, 3H), 2.8–2.65 (m, 1H), 2.65–2.52 (m, 1H), 2.52–2.38 (m, 2H), 2.38–2.15 (m, 3H), 1.95–1.8 (m, 4H), 1.75–1.55 (m, 1H), 1.45–1.1 (m, 44H), 0.87 (t, 3H, J = 6.6 Hz), 0.81 (t, 3H, J = 7.0 Hz); HR-FABMS (*m*/*z*) $[M+H]^+$ calculated for $C_{40}H_{80}NO_8$, 702.5884, found 702.5853.
17. The effect of **2b** in several animal models of autoimmune disease is currently under investigation and will be reported elsewhere.