# Efficient, one-pot syntheses of biologically active α-linked glycolipids†‡

**Wenjun Du, Suvarn S. Kulkarni and Jacquelyn Gervay-Hague***

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**Per-*O*-silylated galactosyl iodides undergo α-glycosidation with fully functionalized glycolipids producing biologically relevant conjugates.**

In 1993, six novel galactosyl ceramides with unique α-glycosidic linkages were isolated from the marine sponge *Agelas mauritianus* near Okinawa, Japan.[1,2] These compounds showed highly potent anti-tumor activities, which prompted various synthetic studies.[3] Among the many analogues synthesized, KRN7000 (Fig. 1) was found to be the most potent[4] and extensive mechanistic studies indicated that the anti-tumor activity resulted from CD1d-dependent natural killer T-cell (NKT) stimulation.[4,5]

CD1d is a member of the CD1 family of proteins, which present lipid antigens to NKT cells to activate the immune response. The current model of activation suggests that CD1d recognizes KRN7000 and the binding complex interacts with the T-cell receptor (TCR) of NKT cells, stimulating the release of two major cytokines known as IFN-γ and IL-4.[6–8] These cytokines are members of the T helper 1 (Th1) and T helper 2 (Th2) cytokine families, respectively. Studies suggest that cytokine production can be tuned by altering the ceramide structure, offering the opportunity to elicit selective Th1 and Th2 secretion.[9–12] For example, Wong and co-workers recently identified an aryl containing fatty acyl chain analog of α-galactosyl ceramide that increases IFN-γ secretion relative to IL-4, and this compound has become a prototype for adjuvant development.[13]

Numerous efforts have been invested in the syntheses of α-GalCer analogs to gain access to these biologically important compounds in pure form for biological and biomedical studies. Arguably, the biggest hurdle in the synthesis is the glycosylation reaction. Achieving chemo- and stereoselectivity requires multi-step protections and deprotections, which consequently lower synthetic efficiency. Moreover, commonly employed glycosyl fluorides[14–17] and trichloroacetimidates[16,18–20] typically afford moderate yields (30–60%) and purification of the products can be complicated by the formation of α/β mixtures. The use of other glycosyl donors such as bromides,[21] thiogalactosides,[19] and phosphites[22] has also been attempted but without significant improvement.

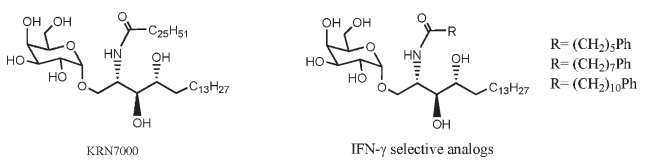


**Fig. 1** Analogs of naturally occurring α-GalCer from *Agelas mauritianus*.

Recent studies in glycosyl iodide chemistry have led to important advances in achieving high stereoselectivity, but there is still room for improvement.[23] For example, reactions of per-*O*-benzylated galactosyl iodide with an azido sphingosine under *in situ* anomerization conditions exclusively produce the α-anomer in over 90% yield (Fig. 2). An azido group is used in place of the amide because, if left intact during the glycosylation, the amide deactivates the primary hydroxyl of the acceptor through unfavorable hydrogen bonding interactions.[24,25] While azide replacement offers a viable solution to this problem, it also requires four additional steps to convert the amine of sphingosine into an azide and, after glycosidation is complete, the azide must then be reduced and conjugated to the fatty acid of interest.

An attractive alternative strategy employs fully functionalized ceramide acceptors, avoiding tedious protection and deprotection steps. Here, we report a streamlined synthesis of bioactive glycolipids using a transiently protected galactosyl iodide donor. The use of per-*O*-silylated galactosyl iodide allows direct glycosidation of fully functionalized lipid components without the need for any protection, providing what is essentially a one-pot synthesis of α-GalCer analogs.

Several attempts to directly incorporate ceramide acceptors have been reported. The nature of the protecting group on the ceramide moiety has significant impact upon the outcome. For example, Wang and co-workers reported that α-selectivity is achieved with *O*-benzoyl protecting groups, whereas β-selectivity is observed with *O*-benzyl protecting groups.[18] Similarly, TBS-protected ceramide

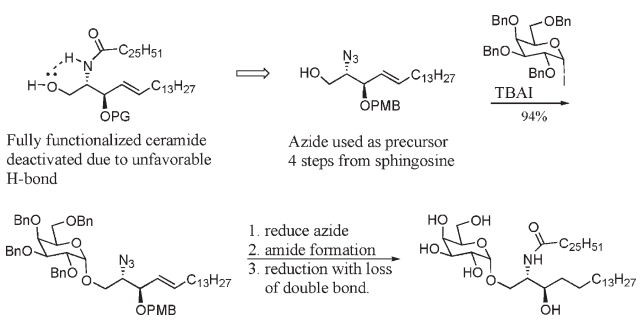


**Fig. 2** First generation synthesis requires multiple protection–deprotection steps.

*Department of Chemistry, University of California Davis, One Shields Avenue, Davis, CA, 95616, USA. E-mail: gervay@chem.ucdavis.edu; Fax: +1 530 754 6915; Tel: +1 530 754 9557*

† Dedicated to Professor Michael E. Jung on the occasion of his 60th birthday.

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acceptors afford coupled products in moderate yields and selectivities depending upon the donor.[16] We were primarily interested in utilizing unprotected acceptors but the only example reported in the literature involved tin activation of a glycosyl fluoride, yielding a complex mixture and poor yields of the desired compound.[14]

Glycolipids **1–3** (Fig. 3) were targeted in order to test the generality of our strategy. Compound **1a** (BbGL-II) was recently isolated from a bacterial source and has led to speculation that the glycolipids isolated from marine sponge may originate from bacteria.[26] Compound **1c** is a saturated analog of **1a**. Recent SAR studies indicate that **1b**, with a reversed arrangement of fatty acid chains, exhibits the most potent immunogenic activity amongst the BbGL-II analogs.[27] Compounds **2** and **3** are analogs of KRN7000 with shorter ceramide lipids and differences in unsaturation. As mentioned earlier, lipid length can bias cytokine production, leading to improved clinical results with lower toxicity in certain disease treatments.[9,11] The most direct synthetic route to these targets would employ fully functionalized lipid components. Although the ceramide acceptors would be deactivated, we were hopeful that a highly reactive glycosyl iodide could overcome this barrier and that a combination of sterics and donor reactivity would make selective protection of the acceptor alcohols unnecessary.

From our previous studies, we knew that per-*O*-benzyl galactosyl iodide would not undergo glycosylation with ceramide acceptors, but recent investigations in our lab suggested that per-*O*-silylated glycosyl iodides are orders of magnitude more reactive.[23] There are several other advantages to engaging per-*O*-silylated donors in synthetic protocols. First, they are readily prepared on a large scale, and the iodide can be generated quantitatively upon reaction with trimethylsilyl iodide (TMSI).[28] Finally, deprotection of the trimethylsilyl group requires only mild acidic conditions, which does not affect the glycosidic linkage. Taken together, the prospects for being able to achieve a highly convergent synthesis of α-Gal analogs appeared favorable.

The synthesis began with **4**, which was reacted with TMSI to give galactosyl iodide **5**. The donor was then added to the acceptor, which was premixed with tetrabutylammonium iodide, a promoter that accelerates the reaction and determines α-stereoselectivity through *in situ* anomerization.[29] After the indicated time, the solvent was evaporated and the remaining residue was subjected to hydrolysis using acidic resin in methanol to give the final products **1a**, **1b**, **2** and **3** (Scheme 1). The results of the

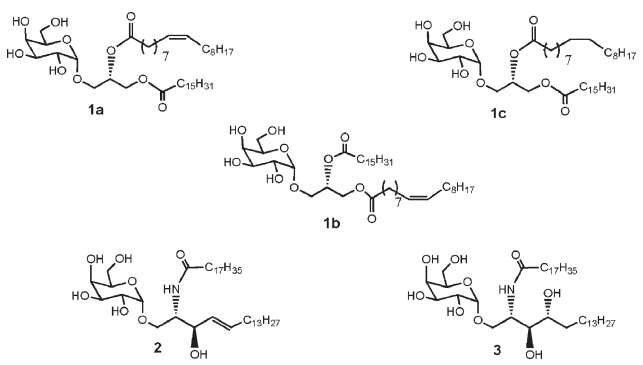
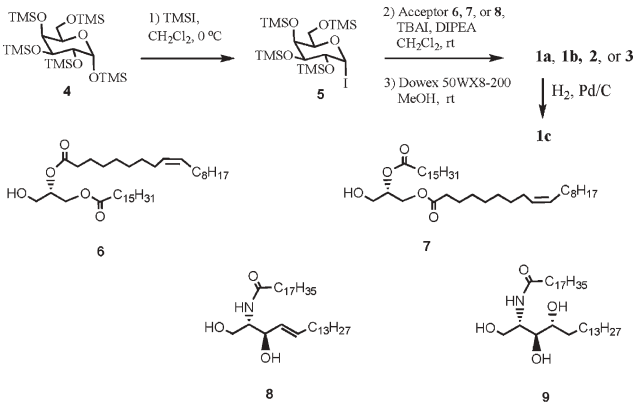



**Fig. 3** Targeted bioactive glycolipids.




**Scheme 1** Synthesis of target compounds.

three-step, one-pot procedure are shown in Table 1. It is notable that this methodology tolerates unsaturation in the fatty acid side-chains; in contrast to benzyl-protected donors, which require hydrogenation for deprotection. To illustrate the point, compound **1a** was readily converted to a saturated analog (**1c**) by hydrogenation of the double bond.

Reactions with the glyceride acceptors **6** and **7**[30] were more facile than with the ceramide acceptors. This is likely due to the fact that **6** and **7** do not possess the unfavorable hydrogen bonding interactions that plague **8** and **9**. Although all the reactions were α-selective, some β-product was observed with acceptor **6** when using benzene as the solvent. A simple solution to this problem was provided by conducting the reaction at room temperature and switching the solvent to $CH_2Cl_2$. In a similar fashion, glyceride **7** reacted to give **1b** in 72% yield (entry 3) and ceramide **8** afforded **2** (77%) uneventfully (entry 4).

More problematic was acceptor **9**,[16] which initially gave only low yields (20–30%, entries 5 and 6). We attempted various reaction modifications including changing solvent, reaction temperature and ratios of donor to acceptor. Despite all these attempts, significant improvements were not forthcoming. We did observe solubility differences between **8** and **9**, which led us to consider micelle formation as a contributing factor. Since the side chain of **9** is saturated, it is able to pack better than **8**. At this point, we attempted microwave assisted glycosylation between **5** and **9** (Table 2). Under low power (25–35 W), the yield was not significantly improved (entries 1–4), although the acceptor appeared to be soluble under these conditions. Increasing the reaction temperature did not improve the yield either. However, much to our delight, a 67% yield of **3** was obtained when performing the reaction at 120 °C for 1.5 h at 225 W (entry 5). We do not fully understand the reasons for the improvement but it is reasonable to suggest that microwave energy may disturb lipid packing making the acceptor more accessible for glycosidation.

**Table 1** Results of one-pot synthesis

| Entry | Acceptor | Product | Solvent | Conditions | α : β ratio (yield) |
|---|---|---|---|---|---|
| 1 | **6** | **1a** | Benzene | 65 °C, 24 h | 10 : 1 (89%) |
| 2 | **6** | **1a** | $CH_2Cl_2$ | rt, 24 h | α only (81%) |
| 3 | **7** | **1b** | $CH_2Cl_2$ | rt, 36 h | α only (72%) |
| 4 | **8** | **2** | $CH_2Cl_2$ | rt, 48 h | α only (77%) |
| 5 | **9** | **3** | Benzene | 65 °C, 48 h | α only (20%) |
| 6 | **9** | **3** | $CH_2Cl_2$ | rt, 48 h | α only (30%) |

**Table 2** Microwave results for the reaction between **5** and **9**

| Entry | Donor | Solvent | Reaction conditions | Yield (%) | α : β ratio |
|---|---|---|---|---|---|
| 1 | 3 eq. | Benzene | 80 °C, 1.5 h, 25 W | 20 | α only |
| 2 | 3 eq. | $CH_2Cl_2$ | 80 °C, 1.5 h, 30 W | 30 | α only |
| 3 | 1 eq. | $CH_2Cl_2$ | 100 °C, 1.5 h, 35 W | 30 | α only |
| 4 | 1 eq. | $CH_2Cl_2$ | 120 °C, 1.5 h, 37 W | 37 | α only |
| 5 | 1 eq. | $CH_2Cl_2$ | 120 °C, 1.5 h, 225 W | 67 | α only |

In summary, we report highly efficient, one-pot syntheses of biologically active α-GalCer and BbGL-II analogs using a combination of a TMS-protected glycosyl iodide donor and an unprotected ceramide or glyceride acceptor. The method is highly efficient, yielding exclusively the α-isomer and providing rapid access to biologically relevant compounds of wide interest. We also demonstrate that microwave energy can significantly improve reaction yields and shorten the reaction time when saturated ceramides are utilized. Further application of these methods to branched oligosaccharides is currently under study in our laboratory.

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30 Acceptor **6** is commercially available, **7** is prepared from (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol in five steps, and **8** and **9** are prepared from sphingosine and phytosphingosine, respectively, through amide bond formation with stearic acid (see supporting information‡).


 