ChemComm

COMMUNICATION

View Article Online
View Journal

Cite this: DOI: 10.1039/c7cc01673e

Received 3rd March 2017,
Accepted 9th May 2017

DOI: 10.1039/c7cc01673e

rsc.li/chemcomm

Borinic acid-catalyzed stereo- and site-selective synthesis of β-glycosylceramides†

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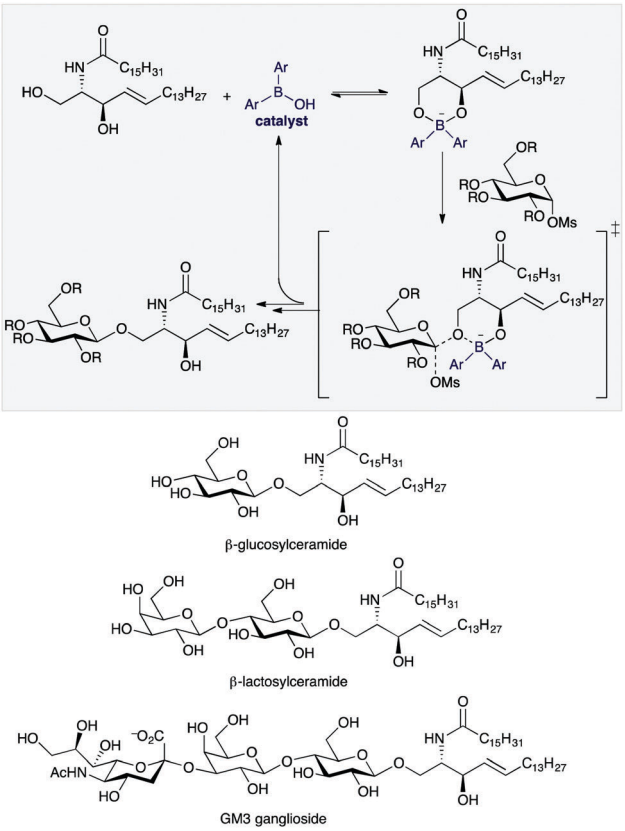
A method for activation of unprotected ceramides towards stereo- and site-selective glycosylation is described. Two-point binding of a diarylborinic acid catalyst to the ceramide accelerates its reactions with 'armed' glycosyl methanesulfonate donors, resulting in the formation of a β-glycosidic linkage at the primary OH group.

Glycosphingolipids are a complex class of biomolecules composed of a ceramide (*N*-acylated sphingosine) lipid linked to a mono- or oligosaccharide, which may be modified with sialic acid or sulfate groups (Scheme 1). They are ubiquitous in mammalian cells, with the ceramide moiety generally residing in the outer leaflet of the plasma membrane, displaying the glycan at the cell surface. Their diverse roles in physiology, human health and disease continue to be elucidated.[1,2] The laboratory synthesis of glycosphingolipids and their analogs has been pursued intensively as a way to access homogeneous material for use in biochemical studies,[3–5] biological probe compounds[6–9] or potential therapeutic agents.[10–12]

One of the challenging aspects of glycosphingolipid synthesis is the selective construction of the glycosidic bond that links the glycan to the primary OH group of the ceramide. Direct couplings of glycosyl donors with ceramides are not straightforward: the reactivity of the primary OH group is attenuated by intramolecular NH···O hydrogen bonding,[13] and aggregation of these amphiphilic acceptors further reduces their reactivity. Azidosphingosine[14] and other *N*-protected sphingosine derivatives[15] show improved reactivity as glycosyl acceptors, and have been employed in numerous studies, often with protection of the secondary OH group. Couplings of unprotected ceramide and phytoceramide with per-*O*-silylated glycosyl iodides have been shown to provide efficient and direct access to α-configured glycolipids.[16] Other approaches include glycosylations of ceramide-derived dialkylstannylene acetals[17] and intramolecular glycosylation through tethering of a glucopyranosyl donor to the secondary OH group of the ceramide moiety.[18] Mutant glycosidase enzymes capable of accelerating the coupling of glycosyl fluorides with sphingosine have been used to generate *lyso* analogs of glycosphingolipids.[19,20]



Scheme 1 Envisioned borinic acid-catalyzed glycosylation of unprotected ceramide, and examples of β-configured glycosphingolipids.

Here, we show that diphenylborinic acid can be employed as a catalyst for site- and β-selective couplings of an unprotected ceramide with glycosyl methanesulfonates (mesylates). This work builds on our recent discovery that diarylborinic acid catalysts are able to activate acceptors containing 1,2- or 1,3-diol groups

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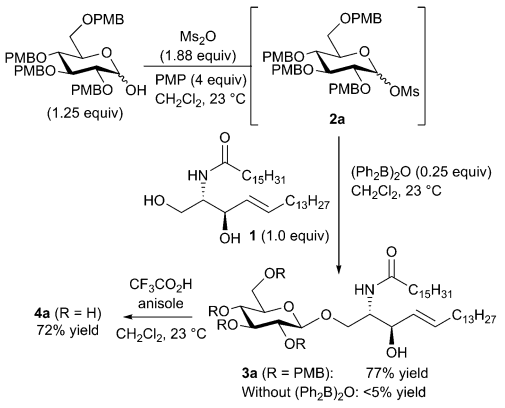
† Electronic supplementary information (ESI) available: Synthetic protocols, characterization data and copies of NMR spectra. See DOI: 10.1039/c7cc01673e

towards reactions with glycosyl mesylates.[21] These glycosylations gave high regioselectivity for the primary position of a 1,3-diol group, generated 1,2-*trans*-configured linkages using mesylate donors lacking protective groups capable of anchimeric assistance, and took place under mild, homogeneous reaction conditions. Each of these factors suggested that borinic acid catalysis with glycosyl mesylate electrophiles could be useful for the challenging couplings of unprotected ceramides, as depicted in Scheme 1. This approach differs from the arylborinic acid-catalyzed coupling of glycal epoxides and protected phytosphingosine derivatives that was reported recently by Takahashi and Toshima:[22] the latter reaction generates α-configured glycolipids through intramolecular aglycon delivery mediated by the boron catalyst, whereas the present method involves activation of the unprotected acceptor through two-point binding, and was expected to deliver products of β-configuration through an associative mechanism.

The coupling of *N*-palmitoyl-D-*erythro*-sphingosine (**1**) and donor **2a** was attempted under the previously optimized conditions for borinic acid-catalyzed reactions of glycosyl mesylates (Scheme 2). *para*-Methoxybenzyl (PMB) ethers were chosen as 'arming'[23] protective groups that could be removed without concomitant reduction of the carbon–carbon double bond of the ceramide. Mesylate **2a** was generated *in situ* from the corresponding hemiacetal by treatment with methanesulfonic anhydride (Ms_2O) in the presence of 1,2,2,6,6-pentamethylpiperidine (PMP). We have shown that under these conditions, MsO^--promoted anomerization is relatively rapid, with the α-anomer being favored at equilibrium.[21] Using diphenylborinic anhydride‡ (0.25 equiv., corresponding to a 50 mol% loading of diphenylborinic acid) as catalyst in dichloromethane at 23 °C, the protected β-glucosylceramide derivative **3a** was obtained in 77% yield (Scheme 2). The stereoselectivity of this glycosylation was at least 19 : 1 β : α, as judged by analysis of the unpurified reaction mixture by ^{1}H NMR spectroscopy. In the absence of catalyst, the yield of **3a** was less than 5%: the reaction mixture consisted primarily of unreacted **1** and the hemiacetal resulting from hydrolysis of mesylate **2a**. The small amounts of glycoside product formed in the uncatalyzed reaction were primarily α-configured, as judged by analysis of the HSQC and COSY NMR spectra of the unpurified reaction mixture (see the ESI†). We have previously found that glycosyl mesylates show appreciable uncatalyzed reactivity with acceptors having free primary or secondary OH groups, giving a modest preference for α-glycoside products. The limited solubility of **1** in CH_2Cl_2, as well as deactivation of the OH groups by intramolecular hydrogen bonding, likely contribute to its low reactivity in the absence of catalyst.



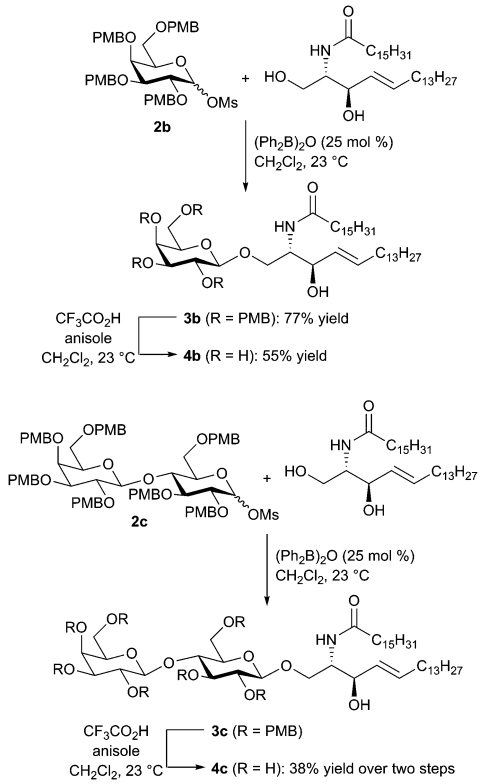
Scheme 2 Synthesis of β-glucosylceramide.

In addition to the rate acceleration relative to the uncatalyzed reaction, the β-selectivity of the borinic acid-promoted coupling of **1** with an armed glycosyl donor is a noteworthy observation. With very few exceptions,[24] couplings of sphingosine and phytosphingosine derivatives with benzyl ether-protected glycosyl donors yield primarily α-glycosidic products. Ester protection of the 2-OH group of the glycosyl donor is the primary strategy for obtaining β-glycosides. This approach requires that the amount of orthoester side product be minimized, either by judicious selection of carbonyl protective groups (*e.g.*, pivaloyl,[25] chloroacetyl[26]) or by employing conditions that promote the rearrangement of orthoesters to glycosides.[17] The ability to generate β-glycosylceramides without relying on neighboring group participation may offer increased flexibility in the design of protective group strategies to access this class of targets. Deprotection of the PMB groups of compound **3a** was accomplished by treatment with trifluoroacetic acid in the presence of anisole as a carbocation scavenger,[27] yielding β-glucosylceramide **4a**.

β-Galactosylceramide **4b** and β-lactosylceramide **4c** were prepared in a similar way, by diphenylborinic acid-promoted coupling of **1** with the corresponding glycosyl mesylates, followed by acid-mediated cleavage of the PMB ether groups (Scheme 3). The separation of protected β-lactosylceramide derivative **3c** from the hemiacetal resulting from donor hydrolysis was challenging, and so the overall yield of **4c** over the two-step process is reported. It should be noted that the cleavage of the PMB groups was quite efficient: the modest yields of the unprotected glycosylceramides reflect the challenging purification of these amphiphilic glycolipids.

In conclusion, we have shown that diarylborinic acid catalysis can be used to effect the otherwise challenging direct glycosylation of *N*-palmitoyl-D-*erythro*-sphingosine. The organoboron-catalyzed couplings with PMB-protected glycosyl mesylate donors take place at room temperature under mild, homogeneous conditions. They deliver the β-1,1′-linkages characteristic of the major classes of mammalian glycosphingolipids, without protection of the ceramide moiety and using armed glycosyl donors lacking protective groups capable of anchimeric assistance. The catalyst loadings are relatively high, resulting in fewer than two turnovers, but Ph_2BOH is significantly less valuable than the substrates (and the products) and its separation from the products is generally straightforward. The ability to conduct direct glycosylations of unprotected ceramides may lead to new opportunities to develop convergent syntheses of glycolipids and their derivatives. These results further illustrate the utility and versatility of borinic acids as promoters for glycosylation reactions of complex partners.[28,29]

This work was supported by NSERC (Discovery Grant and Canada Research Chairs Programs), the Canada Foundation for Innovation (projects #17545 and #19119), and the Ontario Ministry of Research and Innovation. Dr Sean Liew (Yudin group, University of Toronto) is acknowledged for contributions to preliminary experiments related to this study. Dr Darcy



Scheme 3 Synthesis of β-galactosylceramide (**4b**) and β-lactosylceramide (**4c**).

Burns is gratefully acknowledged for assistance with NMR spectroscopy.

Notes and references

‡ In a previous study, we found that $(Ph_2B)_2O$ is the optimal catalyst for activation of 1,3-diol groups in carbohydrate-derived substrates, whereas an oxaboraanthracene-derived borinic acid provides superior results for activation of *cis*-1,2-diol groups (ref. 21). Consistent with this trend, $(Ph_2B)_2O$ provided higher activity that the oxaboraanthracene-derived catalyst for activation of the 1,3-diol group in a ceramide acceptor.

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