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Squaryl Group Modified Phosphoglycolipid Analogs as Potential Modulators of GPR55

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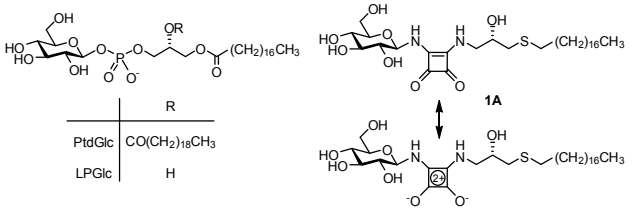
Lysophosphatidyl glucoside (LPGlc) is a structurally unique glycolipid that acts as a guidance cue for extending axons during central nervous system development by activating the class A G protein coupled receptor (GPR) 55 of spinal cord sensory axons. GPR55 not only plays an important role during development, but is also implicated in many disease states, rendering molecules that target GPR55 of widespread interest. In this study, we developed synthetic access to a novel class of LPGlc analogues featuring a squaryl diamide group as surrogate for the phosphodiester. We report the facile synthesis of a series of LPGlc analogues, their GPR dependent biological activity and a systematic analysis of the structure-activity relationship in regards to GPR55 modulation. The lead compound featuring identical configuration at all stereocenters compared to natural LPGlc exhibits an activity to repel axons of dorsal root ganglion (DGR) nociceptive neurons.

Phosphatidyl-β-D-glucoside (PtdGlc) is a structurally unique glycolipid (Scheme 1) which was identified in mammalian cells in 2001.[1] The proposed structure[2] was rigorously confirmed by chemical synthesis.[3] Mammalian PtdGlc exhibits a strikingly homogeneous fatty acid composition and is enriched in lipid rafts.[4] More recently, we revealed that PtdGlc is locally converted to lyso-phosphatidyl-β-D-glucoside (LPGlc, Scheme 1), released from radial glia in the developing spinal cord and regulates sensory axon wiring by specifically repelling nerve growth factor (NGF)-responsive dorsal root ganglion (DRG) axons.[5] Extensive screening of G-protein coupled receptors identified GPR55 as a receptor of LPGlc.

GPR55 is a class A G-protein coupled receptor first identified in 1999.[6] It is expressed in various organs[7] including central and peripheral nervous systems, gastrointestinal tract, bone, and immune systems. Initially it was considered as a cannabinoid receptor[8] but more recently lysophosphatidyl inositol[9] and LPGlc[5] have been reported as its endogenous ligands. It has been shown that GPR55 plays important roles in development[5,6] and is implicated in many disease states such as neuropathic pain,[10] cancer,[11] and inflammation.[12] To-date a number of GPR55 agonists and antagonists have been developed,[13,14] but the need for new specific GPR55 ligands remains,[15] for both clinical and research needs.



Scheme 1 Structures of PtdGlc, LPGlc, and a squaryl analogue **1A**.

To develop a new class of GPR55 modulators, we sought an approach based on the recently identified native ligand structure. LPGlc degradation is expected to proceed either via the phosphodiester (PDE) or the fatty acid (FA) ester group. The PDE links the carbohydrate head group with the *sn*-3 position of the glycerol backbone, while the FA ester attached to the *sn*-1 position. Consequently, we envisaged to replace them with appropriate bioisosteres.[16] As a suitable PDE surrogate, we identified the squaryl (SQ) group, which has been exploited as a bidirectional electrophile suitable for glycan conjugation[17] and protein labeling.[18] The migration prone FA ester was planned to be replaced by a thioether to not only increase the stability but also to satisfy the demand for ease and economy of production. In this context, LPGlc FA ester to ether exchange did not abrogate the activity of LPGlc, while the amide analogue was completely inactive (Guy, Greimel, et al., unpublished). Additionally, the mannose and galactose analogues of LPGlc are also inactive,[5] strongly suggesting to preserve the equatorial configuration of the C-2 and -4 hydroxy group of the glucose residue. Based on the above rationale, compound **1A** (Scheme 1) was envisaged as our lead compound. Sekine and co-workers reported that

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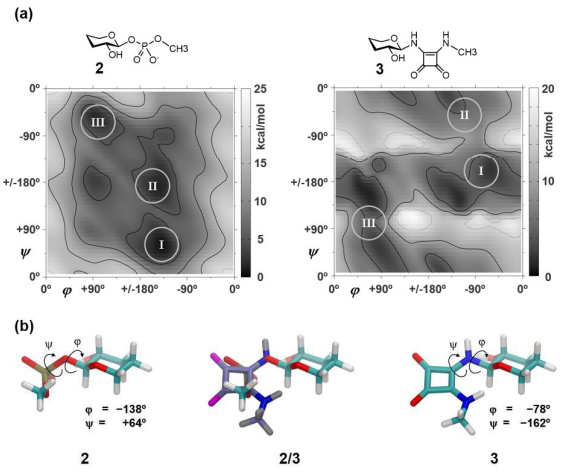
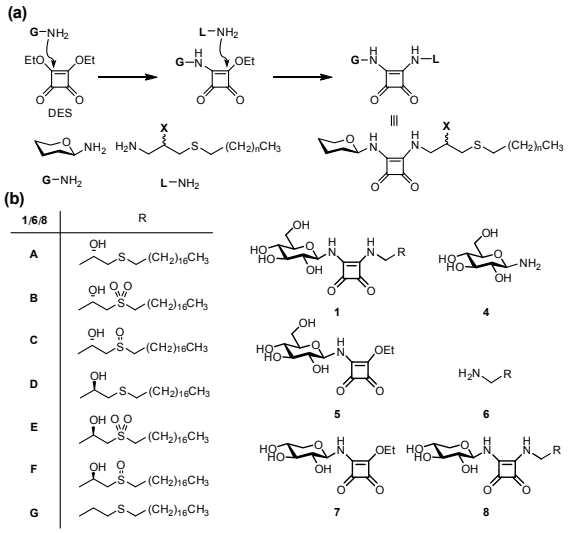


Figure 1 (a) Energy landscape and conformational similarity of equatorial phosphate **2** with equatorial squarate **3** on a pyranose ring, based on QM free energy calculations. (b) The lowest energy conformation of compound **2** is superimposed on the most closest matching conformation of compound **3**. Carbon, cyan or iceblue; oxygen, red or magenta; hydrogen, white or grey; nitrogen, blue; phosphorous, tan.

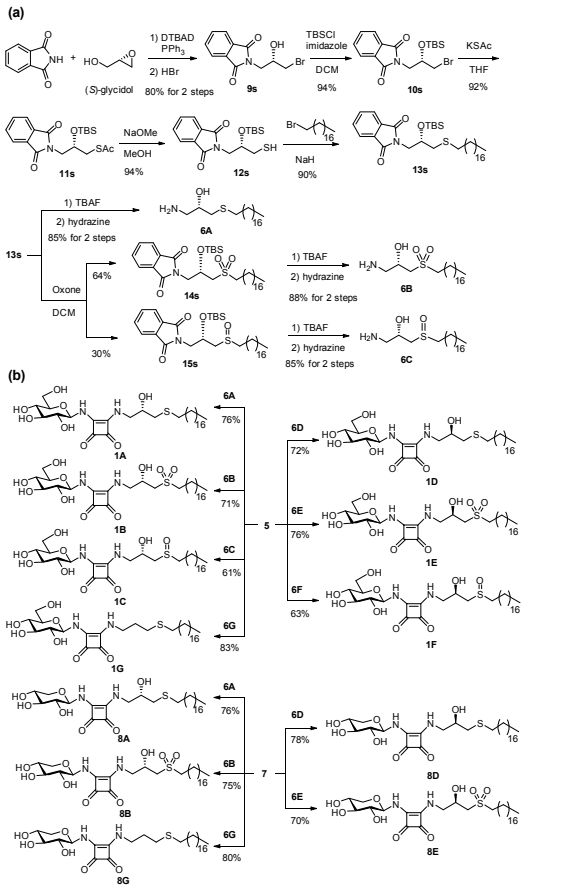
squaryl diamides are polarizable and mimic PDE linkage in DNA oligomers.[19]

To better understand the conformational characteristics of the squaryl group compared to an anomeric phosphate we performed quantum mechanics calculation of model compounds **2** and **3** (Figure 1a). The analysis revealed the presence of three potential energy minima (Figure 1a, labeled I, II and III) in case of an anomeric PDE and a clear rotational barrier of the anomeric bond between $-45^{O}<\varphi<45^{O}$. This is in good agreement with reported NMR results of the LPGlc



Scheme 2 (a) Synthetic strategy, X = -OH (*S*), -OH (*R*) or H. (b) Structures of synthetic blocks and LPGlc analogues.

parent compound phosphatidylglucoside.[20] Interestingly, all potential energy minima of **2** exhibit either gauch^{+} or gauch^{-} conformation of the second inner dihedral of the PDE (Supporting information Figure S4). In case of the squaryl analogue, a considerable number of potential energy minima is observed, with no obvious rotational barrier present in the anomeric linkage between the pyranose analogue and the squaryl group. The conformational space of **3** was ranked against representative conformations of each of the identified potential energy minima in **2**, based on their ability to reproduce one of the carbonyl oxygen and the methyl group positions in **2**. This revealed that **3** can mimic each of the three identified potential energy minima conformations of **2** adequately without the need to attain energetically unfavourable conformations. Superimposition of the lowest energy conformer of **2** associated with potential energy minima **I** and an equivalent conformation of **3**, less than 3 kcal/mol above its respective lowest energy, reveals their high degree of similarity (Figure 1b). Equally matching conformations of **3** with conformations of **2** associated with potential energy minima **II** and **III** exhibited similar energy penalties (Supporting information Figure S5). This suggests that the envisaged glucose residue in combination with the squaryl aglycon is able to attain highly similar conformations compared to LPGlc and thus exhibits a high potential to fit into



Scheme 3 Syntheses of (a) chiral building blocks and (b) LPGlc analogues.

the GPR55 binding pocket.

Our synthetic strategy for the envisaged LPGlc analogues is outlined in Scheme 2a. It is based on three fragments, namely glycosylamine (G-NH_2), squaryl diester (DES), and lipid

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component (L-NH_2). As the sequential coupling of these components can be achieved without additional protection-deprotection, a facile synthesis of various analogues is possible. This strategy also enables access to an array of molecules differing in chain length, stereochemistry, and functional groups. In addition, further diversification is possible by oxidation of the thioether to sulfoxide or sulfone, to allow a broader characterization of the GPR55 binding pocket.

To begin with, glucosylamine **4**[21] readily available from Glc was reacted with diethyl squarate to give mono amide **5**

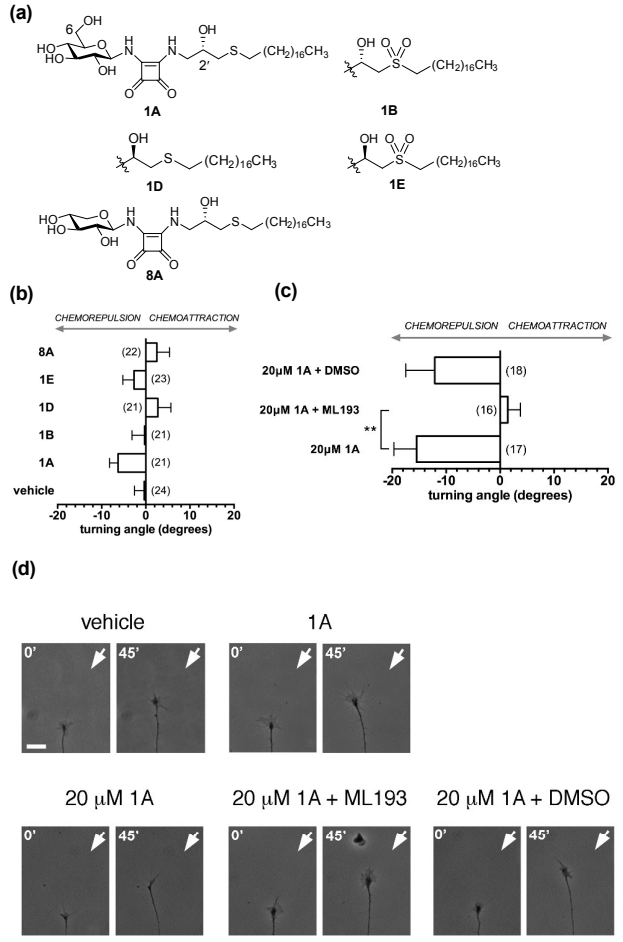

Figure 2 (a) LPGlc analogues used for axon turning assay. (b) Biological activity of squaryl group modified analogues assessed by axon turning assay. Compounds were tested at an in-pipette concentration of 10 μM. Bars show mean turning angle ± SEM, numbers in parentheses indicate the number of axons tested. (c) Effect of GPR55 inhibitor on chemorepulsive response to compound **1A**. Compound **1A** was used at an intrapipette concentration of 20 μM. Treating axons with bath-applied ML193, a specific inhibitor of GPR55, abolished **1A**-induced chemorepulsion. Bars show mean turning angle ± SEM, numbers in parentheses indicate the number of axons tested. **P<0.01, Kruskal-Wallis test. (d) Representative images of axon turning assays using compound **1A**. Numbers in each panel show the minutes after onset of concentration gradient, arrows indicate the source of the gradient. Scale bar, 10 μm. Assay conditions are further described in the supporting information.

(Scheme 2b). On the other hand, a small library of the lipid component (**6A**-**G**) was created (see Supporting Information) as exemplified for **6A** (Scheme 3a). To obtain **6A**-**F** in homochiral forms, they were synthesized from (*S*)- or (*R*)-glycidol as starting materials. Subsequent reaction with **5** gave **1A**-**G** (Scheme 3b). In a similar manner, D-xylose analogues **8A**-**E** were prepared from **7**.

To confirm the relevance of our design of LPGlc analogues, activity of **1A** to initiate axon turning was evaluated in comparison with its diastereomer **1D** and the xylose analogue **8A**, along with sulfones **1B** and **1E** (Figure 2a). Assay was conducted as previously reported[5] with some modifications. Exploratory experiments (Figure 2b) conducted at in-pipette concentration of 10 μM (approximately 10 nM near the tip of axons) (Figure 2b) indicated that the compound **1A** has the most favourable activity to repel axons of DRG nociceptive neurons. Higher chemorepulsive response was observed when the in-pipette concentration was increased to 20 μM (Figure 2c,d), comparable to the native LPGlc[5]. On the other hand, no significant activity was observed for other compounds including the diastereomer **1D** and the xylose analogue **8A**.[22,23] These results indicate the importance of C-6 and C-2' hydroxy groups in order for LPGlc analogues to activate GPR55. The potent analogue **1A** did not affect axon extension (Figure S-2), and was blocked by the specific GPR55 inhibitor ML193,[14c] indicating that it indeed acts on GPR55.

In conclusion, we developed a facile and modular strategy to create novel class of LPGlc analogues. Compound **1A**, exhibiting identical stereoconfiguration of its hydroxy groups as native LPGlc, showed the expected LPGlc-like activity to repel DRG nociceptive axons. This confirmed our initial hypothesis that the squaryl group can act as a bioisostere of anomeric phosphodiesters. Failure of the xylose analogue **8A** to stimulate GPR55, similar to the Gal and Man analogues reported previously, further augments the high preference of the GPR55 binding pocket towards glucose head groups. The inactivity of the sulfone analogues, such as **1B**, suggests the presence of spatial constraints close to the FA ester position. Based on this strategy, systematic synthesis of LPGlc analogues as potential modulators of GPR55 is possible and a more comprehensive structure-activity relationship study along this line is underway.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

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22 For instance, compound **1D** and **8A** did not exhibit meaningful increase in turning angle when the concentration was increased to 20 μM (Figure S-3).
23 Sulfoxide **1C** and **1F** were not tested, because they are mixtures of diastereomers. We discounted the compound **1G** because of its extremely low solubility in DMSO or PBS. Since the compound **8A** was not active, other xylose analogues were not tested.

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