Synthesis and structure-activity relationships of cerebroside analogues as substrates of cerebroside sulfotransferase and discovery of a competitive inhibitor

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Abstract

Metachromatic leukodystrophy (MLD) is a rare genetic disease characterised by a dysfunction of the enzyme arylsulphatase A leading to the lysosomal accumulation of cerebroside sulphate (sulphatide) causing subsequent demyelination in patients. The enzyme galactosylceramide (cerebroside) sulfotransferase (CST) catalyses the transfer of a sulphate group from 3'-phosphoadenosine-5'-phosphosulphate (PAPS) to cerebrosides producing sulphatides. Substrate reduction therapy for arylsulphatase A by inhibition of CST was proposed as a promising therapeutic approach. To identify competitive CST inhibitors, we synthesised and investigated analogues of the substrate galactosylceramide with variations at the anomeric position, the acyl substituent and the carbohydrate moiety, and investigated their structure–activity relationships. While most of the compounds behaved as substrates, α-galactosylceramide 16 was identified as the first competitive CST inhibitor. Compound 16 can serve as a new lead structure for the development of drugs for the treatment of this devastating disease, MLD, for which small molecule therapeutics are currently not available.

1. Introduction

Metachromatic leukodystrophy (MLD) is a rare genetic disease characterised by a dysfunction of the enzyme arylsulphatase A. This defect leads to the lysosomal accumulation of cerebroside sulphate (sulphatide, 1) in various cells such as tubular kidney cells, bile duct epithelia, some neurons, oligodendrocytes and Schwann cells. In particular accumulation in the latter two results in progressive demyelination finally causing lethal symptoms in patients. Recently haematopoietic stem cell-based gene therapy has been shown to be effective in patients in early preclinical states of disease only. Thus, there is an urgent need to develop alternative strategies to treat MLD. One of these strategies is substrate reduction therapy in which galactosylceramide (cerebroside) sulfotransferase (CST; EC 2.8.2.11), the enzyme which synthesises sulphatide, is inhibited. This would diminish the load of accumulated sulphatide in the patient. Such a strategy has been shown to be effective in Gaucher disease, another lysosomal sphingolipid storage disorder. Inhibition of galactosylceramide sulfotransferase has been proposed as a promising new therapeutic strategy for the treatment of MLD. CST catalyses the transfer of a sulphate group from the coenzyme 3'-phosphoadenosine-5'-phosphosulphate (PAPS, 2) to galactosylceramide (3) yielding galactosylceramide sulphate (2) and adenosine-3',5'-bisphosphate (PAP, 4) (Figure 1).

We, therefore, aim at developing CST inhibitors to reduce the biosynthesis of sulphatide to prevent sulphatide aggregation in the central and peripheral nervous system. Sulphation is a widely observed biological reaction conserved from bacterium to human that plays a key role in various biological processes. Deficiencies due to the lack of the ubiquitous sulphate donor PAPS are lethal in humans. A large group of enzymes called sulfotransferases catalyses the transfer reaction of the sulphuryl group of PAPS to the acceptor group of numerous biochemical and xenobiotic substrates. Structure-based sequence alignments based on X-ray crystal structures indicate that the PAPS-binding site is conserved. Therefore, competitive inhibitors for the CST substrate galactosylceramide are expected to have less side effects compared to inhibitors competing with the co-substrate PAPS. However, so far only few weakly potent, non-selective CST inhibitors have been described.

A reaction mechanism between the enzyme, the co-substrate PAPS, and the substrate galactosylceramide has been proposed based on crystal structures. A transition state mimetic might efficiently inhibit the CST-catalysed reaction. In the present study, we synthesised and investigated a series of substrate analogues with the aim to study their structure–activity relationships (SARs) as substrates and to identify competitive inhibitors.
2. Materials and methods

2.1. Commercial compounds

Psychosine was purchased from Sigma (Steinheim, Germany). Galactosylceramide and glucosylceramide were obtained from Matreya LLC (Pleasant Gap, PA); according to the supplier, cerebroside consists of a mixture of saturated or unsaturated fatty acid residues (C16:0, C18:0, C20:0, C22:0, C23:0, C24:0 – C27:0, C24:1 – C27:1) or hydroxyacyl residues (C18:0(2–OH), C20:0(2–OH), C22:0(2–OH), C23:0(2–OH), C24:0(2–OH), C24:1(2–OH), C25:0(2–OH)) and glucosylceramide are a mixture of glucosylceramide with saturated or unsaturated fatty acid residues (C16:0, C18:0, C20:0, C22:0, C23:0, C24:0, C24:1). 3-Phosphoadenosine-5-phosphosulphate (PAPS) was purchased from Bellbrook Labs (No. 2059) in high purity. Other commercial sources of PAPS typically contain significant amounts of PAP and are, therefore, not suitable for the assay. α-Galactosylceramide (KRN7000) was purchased from Avanti Polar Lipids, (Alabaster, AL). β-KRN7000 was synthesised and provided by the laboratory of S. van Calenbergh.

2.2. Chemistry

Precoated Macherey-Nagel SIL G/UV254 plates were used for TLC and spots were examined under UV light at 254 nm and further visualised by sulphuric acid-anisaldehyde spray or by spraying with a solution of (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₆·6H₂O (10 g/L) in H₂SO₄ (10%) followed by charring. Column chromatography was performed on Biosolve silica gel (32–63 μm, 60 Å). NMR spectra were obtained with a Varian Mercury 300 Spectrometer. Chemical shifts are given in ppm (δ) relative to the residual solvent signals, in the case of CDCl₃: δ = 7.26 ppm for H1 and δ = 77.4 ppm for C13 and in the case of pyridine-d5: δ = 8.74, 7.58 and 7.22 ppm for H1 and δ = 149.9, 135.5 and 123.5 ppm for C13. Exact mass measurements were performed on a Waters LCT Premier XE TOF equipped with an electrospray ionisation interface and coupled to a Waters Alliance HPLC system. Samples were infused in a CH₃CN/HCO₂H (1000:1) mixture at 10 ml/min.

2.2.1. General procedure for Staudinger reduction and acylation reaction for compounds 24 and 25

To a solution of azide 23 (400 mg, 0.42 mmol, 1 eq.) in 16 ml of tetrahydrofuran (THF) at room temperature, a 1 M solution of PMe₃ in THF (6.3 ml, 6.3 mmol, 15 eq.) was added dropwise. After stirring for 3 h, 2 ml of H₂O were added and the reaction mixture was allowed to stir overnight at room temperature. Then the solvent was removed under reduced pressure and the residue was co-evaporated with toluene to afford the crude amine. A mixture of the crude amine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 131 mg, 0.84 mmol, 2 eq.) and the appropriate fatty acid (0.63 mmol, 1.5 eq.) in 10 ml of CH₂Cl₂ was stirred for 24 h at room temperature. The reaction mixture was diluted with CH₂Cl₂, washed with H₂O (2/10 ml) and brine (1/10 ml), dried over Na₂SO₄, filtered, and evaporated to dryness. Purification by column chromatography using 10% ethyl acetate in hexane gave the desired amides in the indicated yield.

2.2.1.1. [(15S,25S)-2-Hydroxy-25-[(3S)-3-(3-[1,2-Dihydro-2-oxo-1H-benzo[ghi]quinolin-2-yl]-2-propenyl)oxy]-3,25-dimethoxy-5,7-dioxo-5,6,7,8-tetrahydro-6H-dibenzo[a,d]cyclohepten-2-yl]methan-1-yl]pentanamide (24).

Figure 1. Sulphatide synthesis by CST: galactosylceramide (cerebroside) is converted to sulphatide by CST in the presence of PAPS as sulphate donor.

Figure 2. Chemical structures of aromatic dyes known as CST inhibitors competing with the co-substrate PAPS.
2.2.2.1. \((1\{5,25,3\})-1-[[2,3-Bis-o-(phenylmethyl)-4,6-O-[[5]-phenylmethylene]α-α-galactopyranosyl]oxy]methyl]-2,3-bis(phenylmethylene)heptadecyl](N-Non-adenecanamide) (25). Yield 84%. 1H NMR (300 MHz, CDCl3): \(\delta 0.86-0.92\) (m, 6H, 2 × terminal \(\text{CH}\)), 1.16-1.37 (m, 54H, \(\text{CH}_3\)), 1.37-1.55 (m, 2H, \(\text{CH}_2\)), 1.51-1.72 (m, 2H, \(\text{CH}_2\)), 1.81-1.97 (m, 2H, \(\text{CH}_2\)), 3.50-3.57 (m, 1H, H-4'), 3.74-3.82 (m, 2H, H-1 and H-3), 3.98-3.98 (m, 3H, methyl), 4.35-4.40 (m, 3H, H-2), 4.47-4.63 (m, 3H, methyl), 4.65-4.80 (m, 4H, \(\text{CH}_2\)), 4.86 (d, \(J = 3.1\) Hz, 1H, CHPh), 4.95 (d, \(J = 3.4\) Hz, 1H, \(\text{CH}_2\)), 5.14 (s, 1H, \(\text{CH}_3\)), 5.77 (d, \(J = 8.2\) Hz, 1H, \(\text{CHCl}_3\)), 7.22-7.43 (m, 23H, arom H), 7.48-7.55 (m, 2H, arom H). 13C NMR (75 MHz, CDCl3): \(\delta 14.11\), 22.67, 25.71, 25.83, 29.36, 29.45, 29.71, 29.80, 30.26, 31.91, 36.73, 50.33, 62.93, 68.19, 69.41, 71.72, 71.90, 73.29, 73.81, 74.36, 75.69, 76.15, 77.20, 79.49, 79.85, 99.64, 101.00, 126.31, 127.56, 127.68, 128.22, 128.78, 128.08, 128.29, 128.32, 128.35, 128.84, 137.82, 138.40, 138.53, 138.64, 172.89.

2.2.2.2. \((1\{5,25,3\})-1-[[2,3-Ogalactopyranosyl]oxy]methyl]-2,3-dihydroxyheptadecyl](N-Non-adenecanamide) (27). Yield 42%. 1H NMR (300 MHz, pyridine-d5): \(\delta 0.88\) (t, \(J = 6.4\) Hz, 6H, 2 × terminal \(\text{CH}_3\)), 1.11-1.50 (m, 52H, \(\text{CH}_3\)), 1.60-1.75 (m, 1H, \(\text{CH}_3\)), 1.76-1.99 (m, 4H, \(\text{CH}_2\)), 2.23-2.37 (m, 1H, \(\text{CH}_3\)), 2.46 (t, \(J = 7.5\) Hz, 2H, \(\text{CH}_2\)), 4.30-4.36 (m, 2H), 4.38-4.48 (m, 4H), 4.53 (t, \(J = 6.1\) Hz, 1H), 4.56 (d, \(J = 3.1\) Hz, 1H), 4.63-4.72 (m, 2H), 5.23-5.33 (m, 1H, H-2), 5.59 (d, \(J = 3.8\) Hz, 1H, H-1'), 6.24 (br s, 6H, OH), 8.48 (d, \(J = 8.8\) Hz, 1H, NH). 13C NMR (75 MHz, pyridine-d5): \(\delta 14.86\), 23.50, 27.03, 29.13, 30.17, 30.48, 30.54, 30.58, 30.68, 30.90, 32.68, 34.87, 53.09, 63.10, 68.94, 70.82, 71.47, 72.16, 72.94, 73.41, 77.24, 79.06, 101.81, 157.14, HRMS (ESI) \(m/z\) calculated for \(\text{C}_{43}\text{H}_{80}\text{N}_{10}\text{O}_{10} \ [\text{M} + \text{H}]^+\) 659.4477; found 659.4462.
2.2.5. Synthesis of [(15S,25S,3R)-1-[(α-α-galactopyranosyl-oxy)-methyl]-2,3-dihydroxyheptadecyl]-N-tetracos-15-enamide (22)

The Boc-protected glycoctytopsinogosine 27 (150 mg, 0.26 mmol) was dissolved in CH2Cl2 (20 ml) and 1 M HCl in 90% aqueous AcOH solution (100 μl) was added at room temperature. After 35 min TLC showed incomplete conversion of the starting material and another amount of the HCl solution (100 μl) was added. This process was repeated till TLC (CH2Cl2:MeOH: 8/2) showed full conversion of the starting material. This required a total addition of 800 μl of the HCl solution. The solvents were removed under reduced pressure and the residue was co-evaporated with MeOH (3 × 5 ml). The resulting crude amine (used without further purification) was dissolved in a biphasic mixture of THF (2.5 ml) and saturated aqueous NaOAc (2.5 ml). In a separate flask, nervonic acid (114 mg, 0.31 mmol) was refluxed for 2 h in oxalyl chloride (4 ml) and the crude formed acyl chloride, obtained after evaporation of the solvent by a stream of nitrogen and subsequent drying on high-vacuum, was dissolved in THF (2.5 ml) and added dropwise to the biphasic mixture. The reaction mixture was stirred for 2 h at room temperature and TLC showed complete conversion of the starting material. Next, the aqueous layer was extracted with THF (3 × 15 ml) and the combined organic layer was dried over Na2SO4, filtered and evaporated. The crude residue was purified by column chromatography (10% → 16% MeOH in CH2Cl2) furnishing final compound 22 (79 mg, 37%) as a pale solid. 1H NMR (300 MHz, pyridine-d5): δ 0.86 (dt, J = 6.7, 5.4 Hz, 3H, terminal CH3), 1.15–1.49 (m, 54H, CH2), 1.55–1.74 (m, 1H, CH2), 1.74–1.98 (m, 4H, CH2), 2.07–2.17 (m, 4H, CH2), 2.21–2.35 (m, 1H, CH2), 2.43 (t, J = 7.4 Hz, 2H, CH2), 4.21–4.37 (m, 2H, H-3, H-4), 4.38–4.48 (m, 4H, H-1, H-3” and CH2-6”), 4.53 (t, J = 6.3 Hz, 1H, H-5”), 4.57 (d, J = 2.9 Hz, 1H, H-4”), 4.63–4.73 (m, 2H, H-2” and Hb-1), 4.96 (br s, 1H, OH), 5.24–5.33 (m, 1H, H-2), 5.49–5.55 (m, 2H, CH = CH), 5.59 (d, J = 3.7 Hz, 1H, H-1’”), 6.10 (br s, 1H, OH), 6.21–6.79 (m, 3H, 3 x OH), 6.96 (br s, 1H, OH), 8.48 (d, J = 8.8 Hz, 1H, NH). 13C NMR (75 MHz, pyridine-d5) δ 14.77, 23.41, 26.89, 26.99, 28.02, 30.03, 30.11, 30.26, 30.31, 30.38, 30.41, 30.49, 30.60, 30.64, 30.86, 32.58, 32.61, 34.81, 37.28, 51.97, 63.14, 69.13, 70.79, 71.48, 72.09, 72.97, 73.52, 77.17, 102.01, 130.73, 173.77. HRMS (ESI) m/z: calculated for C48H94NO9 [M + H]+ 828.6923; found 828.6942.

3. Results and discussion

3.1. Chemistry

A series of analogues of the natural CST substrate galactocerebroside with variations in the galactose moiety (α- and β-glycosides, substitution of the sugar moiety), in the hydroxylated alkyl chain, and in the fatty acid residue was designed and synthesised. β-KRN7000 21 and α-glycosides 11, 13–16 and 18–21 were prepared as previously described 22–27. The synthetic route to obtain α-galactosylceramides 12, 17 and 22 is depicted in Scheme 1. The required azidoglycoside 23 was obtained through Lewis acid-catalysed glycosidation reaction as previously reported 28. Staudinger reduction and subsequent EDC-mediated acylation with the appropriate fatty acid furnished 24 and 25. Final catalytic hydrogenolysis yielded α-galactosylceramides 12 and 17. To avoid saturation of the cis-double bond of 15-tetraicosanoic acid, the amino group generated after Staudinger reduction was Boc-protected prior to the removal of the benzyl groups to afford intermediate 27. Subsequent removal of the Boc moiety with HCl in acetic acid gave the corresponding psychosine derivative, which was subjected to Schotten–Baumann acylation with 15-tetraicosanoyl chloride to afford glycolipid 22.

3.2. Biological evaluation

The synthesised compounds were studied using a previously developed capillary electrophoresis-based assay, in which the conversion of the co-substrate PAPS, acting as a sulphate donor, to adenosine-3’,5’-bisphosphate (PAP) was measured 18. In addition to the natural substrate galactosylceramide 3, which contains a...
β-galactosyl residue and is present in nerve tissues as a constituent of myelin, the corresponding β-glucosyl derivative glucoscerbroside (9) was investigated, which is mainly found in liver and spleen. As another naturally occurring sphingolipid psychosine (galactosyl-β-sphingosine, 8) was investigated, which is a cytotoxic derivative of galactosylceramide (3) that lacks its fatty acid residue. Moreover, 13 glycolipids, four of which (12, 17, 20 and 22) are new compounds, not previously described in literature, were investigated as artificial substrate analogues and/or competitive inhibitors of CST (Table 1). Enzyme kinetic parameters (Michaelis–Menten constant and maximal velocity) were determined for all (artificial) substrates for which significant conversion of >20% as compared to the natural substrate (set at 100%) was observed.

3.3. Structure–activity relationships

3.3.1. Substrates

The natural substrates of CST in nerve tissues are β-galactosylceramides (3) which are converted into 3-O-sulphogalactosylcerebroside that constitutes about 4% of total myelin lipids. They are sphingolipids consisting of (i) a sphingosine residue, (ii) a β-galactose, and (iii) a fatty acid residue attached via an amide linkage. Natural galactosylceramide (3) may contain different saturated or unsaturated (hydroxy)fatty acid residues with chain lengths mostly between C18 and C27, frequently C24. Galactosylceramide displayed a \( K_m \) value of 60.3 \( \mu \)M determined in our recently developed capillary electrophoresis-based assay and was the best substrate of all compounds investigated in the present study. The corresponding β-glucosylceramide (9) were shown to be much weaker substrates with only 19% conversion compared to that of 3 (set at 100%) determined under the same conditions. Another natural sphingolipid that had previously been shown to be a substrate of CST is psychosine (8) which is lacking the fatty acid residue of galactosylceramides (3). Psychosine was still efficiently sulphurylated by the enzyme showing an only moderately reduced \( K_m \) value of 103 \( \mu \)M in the same assay. A synthetic galactosylceramide analogue, β-KRN7000 (10), in which the double bond of the sphingosine core structure is hydrated and, therefore, contains an additional hydroxy group, led to reduced conversion (42% compared to 100% for galactosylceramide) and an increased \( K_m \) value of 550 \( \mu \)M (compared to 60 \( \mu \)M for galactosylceramide) determined under the same conditions. Interestingly, the \( \alpha \)-galactoside anomer of compound 10, KRN7000 (18) showed about the same conversion rate and \( K_m \) value indicating that for this series of more polar, hydrated sphingosine-derived synthetic lipids, the enzyme did not discriminate between \( \alpha \)- and β-glycosidic configuration. Thus, we investigated further \( \alpha \)-galactosyl-lipids (11–17, 19–22) derived from KRN7000 (18) mainly with modification of the fatty acid residue. KRN7000 (18) had previously been found to display immunostimulatory and antitumor activity in several in vivo models, and was advanced to clinical trials.

Using the artificial CST substrate KRN7000 (18) as a lead structure, we modified the fatty acid amide moiety. Compound 21, which lacks the fatty acid residue, was not well accepted as a substrate in this series (19% conversion). We subsequently investigated in a systematic manner the introduction of saturated fatty acid residues with increasing chain length. Compound 11 having a short fatty acid residue, namely acetyl, was tolerated as a substrate (40% conversion) confirming that a long fatty acid residue is not required for interaction with the enzyme and acceptance as a substrate. Probing the optimal length of the fatty acid residue led to the following result: C2 (acetyl, 11), C5 (12), C8 (13), C11 (14) and C19 (17) were about similarly good substrates (Table 1). Medium chain fatty acid residues appeared to be less well tolerated than some shorter chain analogs. In particular, palmitic acid (C16, 16) showed a sulphurylation rate below 10%. Interestingly, further increase of the chain length in compound 18 (C26) led to the best substrate in this series of \( \alpha \)-galactosides with 51% conversion. Aromatic substitution in fatty acid amide analogue 19 was tolerated (compare 19 with 13 and 14), while the C24-fatty acid containing a cis-double bond was not a very good substrate. A bulky aromatic substitution on the sugar moiety in 20 strongly reduced the conversion rate (compare 20 with 18). Typical Michaelis–Menten curves are shown in Figure 3 for selected substrates (10, 11, 13 and 18).

3.3.2. Inhibitors

Substrate analogues that were not or only poorly converted by CST with a conversion rate below 20% as compared to galactosylceramide...
| Compound | Structure | Percent conversion<sup>a</sup> at 100 μM | $K_m$<sup>a</sup> (μM) | $V_{max}$<sup>a</sup> (μmol/min/mg protein) | $k_{cat}/K_m$<sup>a</sup> |
|----------|-----------|---------------------------------------|------------------------|----------------------------------------|----------------------|
| β-Glycosides | | | | | |
| 3 Galactosylceramide<sup>b</sup> | | 100%<sup>c</sup> | 60.3<sup>18</sup> | 0.0738<sup>18</sup> | 123<sup>18</sup> |
| 8 Psychosine | | n.d. | 103<sup>18</sup> | 0.105<sup>18</sup> | 102<sup>18</sup> |
| 9 Glucosylceramide<sup>b</sup> | | 19% | n.d. | n.d. | n.d. |
| 10 β-KRN7000 | | 42% | 550 | 0.113 | 20.5 |
| α-Glycosides | | | | | |
| 11 | | 40% | 907 | 0.1080 | 11.9 |
| 12 | | 30% | 358 | 0.0449 | 12.5 |
| 13 | | 22% | 392 | 0.0584 | 15.0 |
| 14 | | 32% | 493 | 0.0590 | 12.0 |
| 15 | | 20% | n.d. | n.d. | n.d. |
might still be able to bind to the enzyme and thereby act as competitive inhibitors. Therefore, we investigated compounds 9, 16, and 10–22 for inhibition of CST activity using two different substrates, the natural galactosylceramide (3) and the synthetic α-galactoside KRN7000 (18). Initial inhibitor screening was performed at 100 μM concentration. Only derivative 16 displayed measurable enzyme inhibition at this concentration (Table 2).

Subsequently, concentration-dependent inhibition was determined against both substrates, and nearly identical \( K_i \) values were determined against both substrates, 127 μM and 159 μM,

| Compound | Structure | Percent conversion\(^a\) at 100 μM | \( K_m \)(μM) | \( V_{max} \)(μmol/min/mg protein) | \( k_{cat}/K_m \)(a) |
|----------|----------|-----------------------------|-------------|----------------------------------|-----------------|
| 16       | ![Structure](image1) | 9%                          | n.d.        | n.d.                             | n.d.            |
| 17       | ![Structure](image2) | 25%                         | 521         | 0.0519                           | 10.0            |
| 18        | KRN7000  | 51%                         | 438         | 0.0758                           | 17.3            |
| 19       | ![Structure](image3) | 32%                         | 751         | 0.0834                           | 11.1            |
| 20       | ![Structure](image4) | 15%                         | n.d.        | n.d.                             | n.d.            |
| 21       | ![Structure](image5) | 16%                         | n.d.        | n.d.                             | n.d.            |
| 22       | ![Structure](image6) | 19%                         | n.d.        | n.d.                             | n.d.            |

\(^{a}\)Standard errors were typically below 30% of the reported mean values.
\(^{b}\)R: saturated or unsaturated alkyl or hydroxyalkyl residue (C16–C27).
\(^{c}\)Conversion of physiological substrate was set at 100%.
\(^{d}\)\( K_m \) and \( V_{max} \) values are shown in bold.
respectively (see Table 2 and Figure 4). To our knowledge, this is the first reported substrate-competitive inhibitor of CST.

4. Conclusions

In conclusion, we synthesised and investigated selected analogues of the natural CST substrate galactosylceramide (3), which were related to the artificial substrate KRN7000 (18). Our aim was to study structure–activity relationships for substrates of the enzyme, and to identify competitive inhibitors. We obtained detailed SAR information for the CST galactosylceramide binding site by analyzing artificial substrates. Most importantly, a novel, competitive CST inhibitor (16) was identified. Compound 16 can serve as a

![Figure 3](image)

**Table 2.** CST inhibitory activity of galactocerebroside analogues

| Compound | Structure | Kᵢ ± SEM (µM) versus galactosylceramide (% inhibition at 100 µM) | Kᵢ ± SEM (µM) versus KRN7000 (% inhibition at 100 µM) |
|----------|-----------|---------------------------------------------------------------|-----------------------------------------------------|
| β-Glycosides | 9 Glucosylceramide | (9%) | (−5%) |
| | R= saturated or unsaturated alkyl or hydroxyalkyl residue | | |
| α-Glycosides | 16 | 127 ± 12 | 159 ± 55 |
| | R= saturated or unsaturated alkyl or hydroxyalkyl residue | | |
| | 20 | (4%) | (1%) |
| | | | |
| | 21 | (4%) | (−30%) |
| | | | |
| | 22 | (−7%) | (1%) |
lead structure for optimisation to obtain potent competitive CST inhibitors, which are urgently needed for the treatment of MLD. Our future goal is to develop CST inhibitors for substrate reduction therapy to help MLD patients to survive this devastating genetic disease.

Disclosure statement
The authors declare no conflict of interest, financial or otherwise.

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