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Antileishmanial activity of sp^2-iminosugar derivatives

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A series of sp^2-Iminosugar-type glycomimetics bearing *S*-linked pseudoglycoside substituents (sulfide, sulfoxide and sulfone derivatives) has been synthesized and evaluated as new potential drugs against the protozoan parasite *Leishmania*, responsible of leishmaniasis, the second most relevant parasitic disease after malaria. All the prepared compounds share a bicyclic 5*N*,6*O*-oxomethylidenenojirimycin glycone-like moiety bearing a substitution pattern of configurational complementarity with the natural α-glucosides and incorporate either an *n*-octyl or *n*-dodecyl aglycone-like substituent. Not surprisingly, they behaved as potent to moderate competitive inhibitors of α-glucosidase (inhibition constants, K_i, in the range 1.3 to 447 μM). Evaluation of the antileishmanial activity indicated that the dodecyl pseudoglycosides present a significant antiparasitic activity in intracellular amastigotes of *Leishmania donovani*, the clinically relevant form of the parasite. The antileishmanial effect seems to be associated to the anticancer and proapoptotic activity of the glycomimetics, but not to the α-glucosidase inhibitory efficiency. The (S_S)-configured dodecylsulfoxide derivative **4**, exhibiting the most favourable activity/toxicity profile, was further assayed in combination treatment with miltefosine, the first oral antileishmanial drug, using the fixed ratio isobologram method. The interaction between derivative **4** and 0.1, 0.2 and 0.3 μM miltefosine was classified as synergistic, showing combination indices of 0.78, 0.76 and 0.80, respectively. Additionally, a miltefosine resistant *Leishmania* line and the wild-type strain showed similar susceptibility to derivative **4**. The results illustrate the potential of sp^2-iminosugar pseudoglycosides as promising prototypes for the development of new therapeutic strategies for leishmaniasis.

Introduction

Leishmaniasis is a broad spectrum disease caused by protozoan parasites of the genus *Leishmania*, which are transmitted by the bite of infected sandflies. It is one of the world's most neglected diseases affecting 12 million people in 98 countries, with 350 million people considered at risk of infection and 40000 deaths per year.[1] *Leishmania donovani* is responsible for visceral leishmaniasis (VL) in India subcontinent and East Africa, a disease that is lethal in the absence of treatment. Current leishmaniasis treatment relies exclusively on chemotherapy, such as pentavalent antimonials, amphotericin B, miltefosine, and paromomycin. These first-line drugs have a limited efficacy due to growing resistance, frequent side effects and the high cost of treatment.[2] Therefore, the World Health Organization (WHO) has recommended combination treatment in order to increase the effective life of the available medicines, reducing the treatment duration and cost and the probability of selection of drug-resistant parasites.[3] Miltefosine is an alkylphosphocholine originally developed as an anticancer drug that has become the first oral drug to treat leishmaniasis.[4] Miltefosine monotherapy regimen is well tolerated, except for mild gastrointestinal side effects, although it is potentially teratogenic. Furthermore, experimental resistance to miltefosine is very easily achieved,[5] suggesting the need to introduce new therapeutic strategies to prevent treatment failure. Several miltefosine-containing combined treatments for VL have been conducted with favourable results in India,[6] and others are currently being explored in multiple controlled clinical trials in East Africa.[7] These results strongly suggest that the success of the on going efforts against leishmaniasis will critically depend on the identification of new active molecules that could broaden the current multidrug formulation options.

Iminosugars,[8] nitrogen-in-the-ring carbohydrate mimics (glycomimetics), have been proposed as potential candidates for the development of new antiparasite drugs.[9] These natural or synthetic polyhydroxylated alkaloids can interact with a range of carbohydrate processing enzymes such as

glycosyltransferases, glycosidases and nucleoside-processing enzymes, thereby interfering in many biological processes of medicinal interest.[10] Thus, iminosugar derivatives such as 1-deoxynojimicyn (DNJ) or castanospermine (CS) have been described as immunosuppressive,[11] antitumor[12] and antiviral agents.[13] Recently, Ruhela et al.[14] have reported a new family of bicyclic iminosugars behaving as inhibitors of elongating α-D-mannosyl phosphate transferase of microsomal membranes of *L. donovani*, suggesting that these glycomimetics could be also considered as promising antileishmanial drugs. The broad range of potential activities of iminosugars represents however a limitation for their clinical application, emphasizing the need for developing more selective leads.

Replacement of the endocyclic amine-type nitrogen atom characteristic of classical iminosugars by a sp^2-hybridized pseudoamide-type nitrogen (guanidine, urea, carbamate, isourea, or their thio-analogues) has been shown to afford a new family of glycomimetics (sp^2-iminosugars) with unprecedented abilities to discriminate between different glycosidase isoenzymes.[15] Several sp^2-iminosugars are currently under investigation as pharmacological chaperones for lysosomal storage disorders, including Gaucher,[16] Fabry[17] and G_{M1} gangliosidosis diseases.[18] Interestingly, compounds with pseudoglycoside structure[19] have been found to exhibit antitumor activity, which was ascribed to their ability to interfere with *N*-glycoprotein biosynthesis by inhibiting the neutral endoplasmic reticulum α-glucosidase. The *S*-linked octyl glycoside bicyclic nojirimycin analogue **1** was particularly efficient at this respect, being able to arrest the cell cycle and induce apoptosis in breast cancer cell lines without affecting normal cells.[20] Since several compounds with antileishmanial activity have been shown to act through programmed cell death mechanisms,[21] assaying the potential of sp^2-iminosugar *S*-pseudoglycosides for the treatment of leishmaniasis seemed intriguing. Following our efforts in this field, here we present the synthesis of new sp^2-iminosugar thioglycoside- (**2**), glycosyl sufoxide- (**3**-**6**) and glycosyl sulfone-type (**7** and **8**) derivatives (Figure 1). The compounds were first assayed for their inhibitory activity towards a panel of commercial glycosidases. Next, the antileishmanial activity of derivatives **1**-**8**, was evaluated against promastigotes, axenic and intracellular amastigotes of *L. donovani*. The known[19a] *N*- and *C*-pseudoglycoside derivatives **9** and **10** were also included in this study to check the effect of the nature of the glycosidic linkage in the biological activity. Cytotoxicity against the human monocytic cell line THP-1 and the fibroblast cell line MRC-5 was also assessed in order to exclude those molecules showing unfavourable toxicological profile for further development.

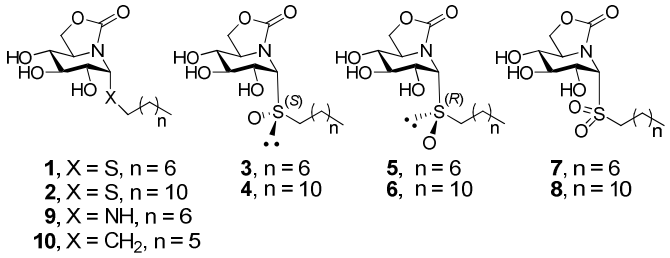
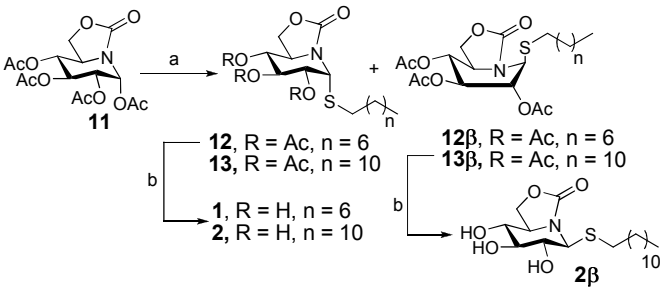


Figure 1. Chemical structure of sp^2-iminosugars evaluated in this study.

Results and Discussion

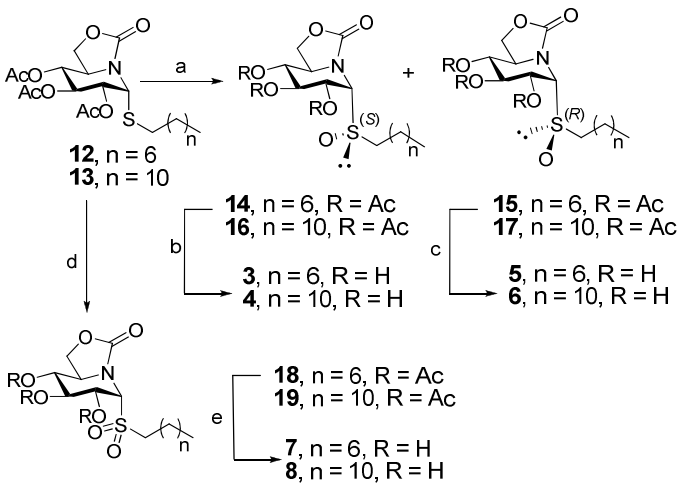
Synthesis

The octyl- and dodecyl-thioglycoside sp^2-iminosugar derivatives **1** and **2** were readily synthesized from the per-*O*-acetylated bicyclic nojirimycin derivative **11**[22] by reaction with commercial 1-octanethiol or 1-dodecanethiol in the presence of boron trifluoride etherate ($BF_3 \cdot OEt_2$)[23] at 0 ºC and conventional deacetylation of the resulting adducts (Scheme 1; overall yield 85%-95%). The stereochemical outcome of this reaction is remarkable, affording in both cases the α-anomer (1*R*) **12** or **13**, respectively, as the major diastereomer (α:β ratio 20:1), in spite of the participating character of the acetyl group vicinal to the pseudoanomeric position. This result underlines the utmost influence of the anomeric effect in the reactivity and stability of sp^2-iminosugars, favoring the axial orientation of pseudoanomeric substituents. Compounds **12** and **13** can satisfy this requirement in the $^4C_1$ chair conformation, whereas the β-anomers (1*S*) **12β** or **13β** have to adopt a skew-boat conformation at the six-membered ring, a less favourable situation, to fulfill the anomeric effect. Compound **13β** was isolated in sufficient amount to allow subsequent conventional deacetylation. The fully unprotected compound **2β** adopted instead the $^4C_1$ conformation in water solution, with the *S*-dodecyl substituent in equatorial orientation, probably due to the expected weakening of the electrostatic contribution to the anomeric effect in polar solvents.



Scheme 1. Synthesis of pseudoalkylthioglycoside iminosugar derivatives **1** and **2**. Reagents and conditions: a, RSH, $BF_3.OEt_2$, DCM, 0 ºC to rt; b, NaOMe (1 M), MeOH, rt.

Oxidation of the acetylated α-pseudoglycosyl sulfides **12** and **13** by treatment with one quivalent of *m*-chloroperoxybenzoic acid (*m*CPBA) at 0 ºC for 10 min[24] afforded the corresponding acetylated glycosyl sulfoxides as 1:1 mixtures of the (S_S) and (R_S) diastereomers at the chiral S-atom (**14** and **15** from **12**; **16** and **17** from **13**, respectively). The optically pure diastereomers could be separated by column chromatography and further deacetylated to afford the target fully unprotected (S_S) and (R_S) octyl- (**3** and **5**) and dodecyl- (**4** and **6**) sulfoxides. When oxidation of the acetylated sulfides **12** and **13** was effected with an excess of *m*CPBA, the peracetylated α-configured sulfones (**18** and **19**) could be isolated in 78-90% yield. Deprotection by conventional deacetylation resulted in the unprotected sulfones **7** and **8** (Scheme 2).



Scheme 2. Synthesis of sulfinyl and sulfonyl iminosugar derivatives **3-6** and **7-8**. Reagents and conditions: a, MCPBA (1 equiv), DCM, 0 ºC, 10 min, 69% (global yield for the octylsulfinyl derivatives), 74% (global yield for the dodecylsulfinyl derivatives); b, NaOMe (1 M), MeOH, rt., 15 min, 85%-93%; c, NaOMe (1 M), MeOH, rt., 30 min, 87%-88%. d, MCPBA (2 equiv), DCM, 0 ºC, 10 min, 78%-90%; e, NaOMe (1 M), MeOH, r.t., 20 min, 83%-84%.

It is well known the importance of chirality in drugs, hence the need of determining the stereochemistry of the chiral sulfur in drugs bearing sulfoxides as they may display different chemical and pharmacological behaviour.[25] Although no single crystals of the diastereomeric sulfoxides **3** and **4** or **5** and **6** suitable for X-ray diffraction could be obtained, some diagnostic chemical shift differences in the respective ^{1}H NMR spectra allowed the tentative assignment of their absolute configuration. Notably, the H-5 resonance was deshielded by 0.2 ppm in derivatives **3** and **4** as compared with **5** and **6**. Literature data on alkyl α-D-glycosyl sulfoxides support then the (S_S) assignment for the first ones, where H-5 and the sulfoxide oxygen would be located in close proximity in the more favourable exoanomeric-type conformation, that is, with C-2 in the ring and the exocyclic methylene carbon in anti disposition.[26a] The lower magnetic nonequivalence (Δδ) of the methylene protons vicinal to the chiral sulfur atom (SOCH_2) for **3** and **4** (35 Hz) as compared with **5** and **6** (70 Hz) is also in agreement with the NMR properties reported in the literature for (S_S) and (R_S) alkyl α-D-glycosyl sulfoxides, respectively.[26b]

Table 1. Inhibition constants (K_i, μM) for pseudoalkylthioglycosides (**1**, **2**), sulfoxides (**3**, **5**, **4**, **6**) and sulfones (**7**, **8**) towards commercial glucosidases.

Glycosidase (source)[a]	**1**	**2**	**3**	**5**	**4**	**6**	**7**	**8**
α-Glcase (ER, yeast)	3.4	1.3	44	14.3	272	447	11.8	6.4
Isomaltase	7.2	36	84	18	636	487	16.5	382
β-Glcase (bovine liver)	60	79	37	214	290	443	126	240

[a] K_i values were determined from the corresponding Lineweaver-Burk plots (see Supporting Information for experimental details).

Glycosidase inhibitory activity

Previous studies on sp^2-iminosugars pointed to a relationship between their ability to inhibit neutral α-glucosidase and their proapoptotic activity. In order to ascertain if α-glucosidase inhibition can be used as a preliminary criterion to select candidates against leishmaniasis, and also to discard broad range glycosidase inhibitors with potential secondary effects, we first evaluated the inhibitory activity of compounds **1-8** against a panel of commercial glycosidases. The corresponding data are summarized in Table 1. None of them showed inhibition towards α-mannosidase (jack beans), β-mannosidase (*Helix pomatia*), α-galactosidase (green coffee) or β-galactosidase (*E. coli*), in agreement with their D-*gluco* configurational pattern. As expected, the pseudoglycosyl sulfides **1** and **2** exhibited strong inhibitory activity against the neutral α-glucosidase II (yeast), with K_i values in the low μM range (1.3 - 3.4 μM), regardless of the length of the aliphatic chain. However, their oxidized analogues bearing the sulfinyl chain displayed remarkable differences not only in activity but also in selectivity depending on the configuration of the new stereogenic centre generated after oxidation. Thus, the (R_S)-octylsulfoxide **5** (K_i = 14.3 μM) was a 3-fold more potent inhibitor against this enzyme than **3** (K_i = 44 μM), which on the contrary was a stronger inhibitor of β-glucosidase (K_i = 37 and 214 μM for **3** and **5**, respectively). Somehow surprisingly, the inhibitory activity dramatically decreased for the dodecyl sulfoxides **4** and **6** (K_i values against α-glucosidase 272 and 447 μM, respectively), whereas both the octyl and dodecyl sufone derivatives **7** and **8** behaved as strong inhibitors of this enzyme.

Antileishmanial activity and cellular toxicity of the novel iminosugar pseudoglycoside inhibitors

Antileishmanial activity of the *S*-pseudoglycoside sp^2-iminosugar compounds **1-8** has been evaluated against promastigotes, axenic amastigotes and intracellular amastigotes of *L. donovani*, using a MTT-based assay, resazurin or luciferin assay, respectively. The previously reported 1-octylamino (**9**) and 1-*C*-octyl (**10**) derivatives (Figure 1) were also included in this study. *Leishmania* has two major life cycle stages: promastigotes, which are easily cultured in suspension, and intracellular amastigotes, which are more difficult to maintain *in vitro* since they require macrophages as host cells with highly acidic intracellular environment. Considered as an

intermediate form, axenic amastigotes are adapted to grow in a medium that mimics the intracellular conditions of macrophages. Cell viability was evaluated by determining the concentration of compound required to inhibit the growth of parasites by 50% (EC_{50}). Neither of the compounds assayed presented significant activity against the extracellular promastigote forms (Table 2); however, compounds with the longer dodecyl aliphatic chain (**2**, **4**, **6**, **8**) showed moderate antileishmanial activity against axenic and intracellular amastigotes, suggesting that the activity of this set of compounds relies specifically on the clinically relevant form of the parasite (Table 2). Except for sulfide **2**, these compounds were more efficient against intracellular amastigotes as compared with axenic amastigotes, which points to some additional host cell-mediated effector mechanisms implied in intracellular parasite killing by the sulfoxide (**4** and **6**) and the sulfone (**8**) pseudoglycosides.[27] A similar scenario has been described for miltefosine activity,[28] highlighting the interest of these sp^2-iminosugar derivatives as promising antileishmanial candidates for further drug development.

The critical influence of the length of the aliphatic chain of the sp^2-iminosugar pseudoglycosides in the antileishmanial activity is remarkable. Thus, the inhibitory effect on intracellular amastigotes was drastically reduced or abolished on going from the dodecyl (**2**, **4**, **6** or **8**) to the octyl pseudoglycoside counterparts (**1**, **3**, **5** or **7**, respectively). Considering that these parasite forms reside and multiply inside the infected mammalian host cells, the observed differences may result from a greater cellular permeability for the more lipophilic dodecyl derivatives. In any case, the data discard a direct relationship between the α-glucosidase inhibition potency and the antileishmanial activity. Indeed, a preliminary assay of the anticancer activity of these compounds towards a panel of cancer cell lines likewise indicated a higher antiproliferative activity for the dodecyl versus the octyl pseudoglycosides (data not shown). It seems therefore that anticancer and antileishmanial activity could be actually linked in this family of glycomimetics, but the previously advanced hypothesis of inhibition of α-glucosidase being at the origin of the biological activity must be taken with caution.[29]

Cytotoxicity of compounds has been tested against the human lung fibroblast cell line MRC-5 and against the human monocytic leukemia cell line THP-1, the host cell used in the assay with intracellular amastigotes. In general, the MRC-5

Table 2. Drug susceptibility profile of iminosugar derivatives for promastigotes, axenic amastigotes and intracellular amastigotes of *Leishmania donovani* and cellular toxicity in THP-1 and MRC-5 cells.[a]

Compound	Promastigotes *L. donovani* Dd8 EC_{50} µM	Axenic amastigotes *L. donovani* HU3 EC_{50} µM	Intracellular amastigotes Dd8 EC_{50} µM, [SI] [b]	THP-1 EC_{50} µM	MRC-5 EC_{50} µM
1	>100	>100	73.31 ± 1.97 [0.79]	122.33 ± 5.79	58.14 ± 12.62
2	87.79 ± 3.47	14.52 ± 1.03	20.48 ± 3.59 [2.10]	70.46 ± 3.75	43.07 ±9.83
3	>100	>100	>100	218.74 ± 0.45	175.54 ± 14.79
4	>100	24.38 ± 1.68	10.80 ± 0.27 [8.74]	118.83 ± 3.37	94.35 ± 20.93
5	>100	>100	>100	161.79 ± 20.27	190.10 ± 0.14
6	>100	60.52 ± 17.35	33.29 ± 7.36 [3.59]	68.41 ± 6.79	119.40 ± 17.91
7	>100	>100	>100	285.26 ± 55.14	159.79 ± 11.75
8	>100	46.64 ± 2.92	19.08 ± 0.84 [3.17]	99.58 ± 12.47	60.23 ± 2.86
9	>100	>100	47.00 ± 8.80 [3.29]	208.69 ±10.32	154.52 ± 5.30

10	>100	>100	>100	203.18 ± 23.56	54.76 ± 6.24
Miltefosine	6.60 ± 1.57	1.40 ± 0.19	0.44 ± 0.08 [130.86]	26.86 ± 3.08	57.58 ± 6.38

[a] Parasites were grown as described in the Experimental section for 72 h at 28 °C (promastigotes) or 37 ºC (axenic and intracellular amastigotes) in the presence of increasing concentrations of compounds. THP-1 and MRC-5 cells were grown as described in the Experimental section for 72 h at 37 °C, in the presence of increasing concentrations of compounds. Cell viability was determined using an MTT-based assay (promastigotes), resazurin assay (axenic amastigotes) or luciferase assay (intracellular amastigotes). Miltefosine was used as the reference antileishmanial agent. Data are means of EC_{50} ± SD from three independent experiments.

[b] Selectivity indices [SI] were calculated by dividing the EC_{50} values for MRC-5 cells by that for intracellular amastigotes.

cells are more susceptible than the THP-1 cells and most compounds show moderate cell toxicity against MRC-5, with EC_{50} values between 50 and 190 μM. Taking into account the mammalian cytotoxicity, compound **4** was the most promising candidate, with a significant activity against intracellular amastigotes of *L. donovani* Dd8 (EC_{50} 10.80 ± 0.27 μM) and a relatively low toxicity against THP-1 and MRC-5 cell lines (EC_{50} 118.83 ± 3.37 and 94.35 ± 20.93 μM, respectively; Table 2).

Finally, we evaluated the activity of compound **4** towards intracellular amastigotes of a previously described *L. donovani* line (M-40R) resistant to miltefosine, the first oral drug against leishmaniasis. [30] In spite of being >29-fold more resistant to miltefosine (EC_{50} >40 μM) relative to the *L. donovani* HU3 wild-type strain (EC_{50} 1.36 ± 0.12 μM), the susceptibility of the M-40R line to compound **4** was similar to the wild-type strain, with EC_{50} values of 20.55 ± 1.78 and 19.19 ± 0.70 μM, respectively.

Drug combination with miltefosine

Taking into consideration the recommendation of WHO with respect to the use of drug combinations in the chemotherapy of leishmaniasis,[3] we next investigated the effect of the combination of the most active sp^2-iminosugar candidate **4** with miltefosine. For that purpose, intracellular amastigotes of *L. donovani* were treated with different concentrations of compound **4** (2, 4, 8 and 16 μM) at fixed concentrations of miltefosine (0.1, 0.2 and 0.3 μM), as described in the Experimental section. Dose-response curves showed that the combined treatment of **4** and miltefosine was more effective at inhibiting the parasite growth as compared with compound **4** alone (Figure 2, Table 3).

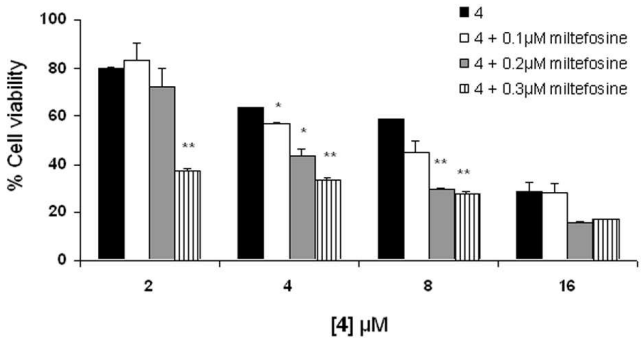


Figure 2. Effects of combination of compound **4** with miltefosine on *L. donovani* intracellular amastigotes viability**.** Intracellular *L. donovani* amastigotes in infected THP-1 cells were grown and exposed to drug pressure for 72 h at 37 °C in the presence of increasing concentrations of compounds **4** (2, 4, 8 and 16 μM) + miltefosine (0.1, 0.2 and 0.3 μM). Parasite viability was determined using the luciferase assay (as described in the Experimental section). Data are means of EC_{50} ± SD from three independent experiments. Significant differences were determined using Student's *t* test (*, $p < 0.01$; **, $p < 0.001$).

The combination of compound **4** with 0.1, 0.2 and 0.3 μM miltefosine induced a decrease of the EC_{50} values from 10.8 μM to 6.4, 3.6 and 1.3 μM, respectively (Table 3). The effect of combination treatment (synergy, additivity or antagonism) was determined by using the classic isobologram method[31] and the combination index (CI) for each **4**:miltefosine combination. Isobolographic analysis (Figure 3) showed that all the interactions of compound **4** with miltefosine were synergistic in all the assayed combinations. CI values of compound **4** combined with 0.1, 0.2 and 0.3 μM miltefosine were 0.78, 0.76 and 0.80 respectively, indicating a moderate synergism.[32]

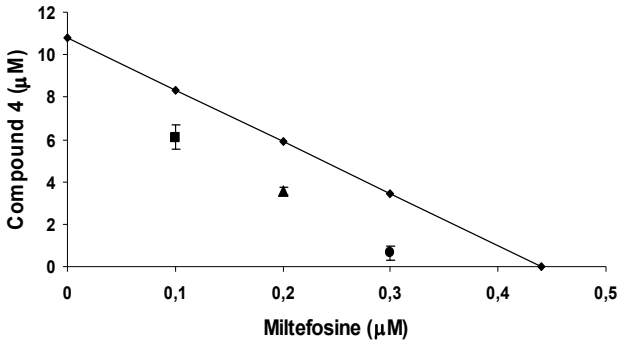


Figure 3. Isobologram analysis for the combinations of compound **4** and miltefosine. The line indicates synergy, additivity or antagonism when the points are located below, on or above the line, respectively. (a) **4** + 0.1 μM miltefosine, (b) **4** + 0.2 μM miltefosine, (c) **4** + 0.3 μM miltefosine. Data are means ± SD of three independent experiments.

We have further evaluated the toxicity of the different combinations of compound **4** with miltefosine in THP-1 and MRC-5 cells. At the concentrations used, the drug combinations were not cytotoxic (data not shown). Overall, the ensemble of results supports that sp^2-iminosugar pseudoglycosides can be considered as promising molecules for

the development of new combination therapies against leishmaniasis. The advantage of combination therapy includes an increased effectiveness of the drug, reduced dosage, decreased toxicity and a delay or prevention on the appearance of drug resistance.

Table 3. Inhibitory concentrations at EC_{50} of the association between compound **4** and miltefosine on *L. donovani* intracellular amastigotes. [a]

Compound		EC_{50} μM
4		10.80 ± 0.27
	+ miltefosine 0.1 μM	6.44 ± 1.05
	+ miltefosine 0.2 μM	3.56 ± 0.16
	+ miltefosine 0.3 μM	1.31 ± 0.19
Miltefosine		0.44 ± 0.08

[a] Intracellular *L. donovani* amastigotes in infected THP-1 cells were grown and exposed to drug pressure for 72 h at 37 ºC in the presence of increasing concentrations of compounds. Parasite viability was determined using the luciferase assay (as described in the Experimental section). Miltefosine was used as the reference antileishmanial agent. Data are means of EC_{50} ± SD from three independent experiments.

Conclusions

In conclusion, the results disclosed in this study provide the first evidence of antileishmanial activity of sp^2-iminosugar derivatives. The possibility of using these compounds in combination therapy with miltefosine is particularly interesting in view of the observed synergistic effect. Although much research is still needed to ascertain the exact mechanism of action, the current body of data suggests a relationship between anticancer (proapoptotic) and antileishmanial activity in this family of glycomimetics. No direct relationship between these biological activities and the inhibition of glycosidases has been established so far, however. Indeed, glycomimetics can potentially interact with a range of additional enzymes and receptors, including glycosyltransferases,[33] lectins[34] and chaperones,[35] that can interfere in cell proliferation and cell death. In any case, the antileishmanial activity and the selectivity towards the intracellular form of the parasite is strongly dependent on the nature of the aglycone-type substituent, underlining the importance of developing diversity-oriented synthetic strategies allowing optimization of non-glycone interactions for specific applications.[36]

Experimental

General Methods

Reagents and solvents were purchased from commercial sources and used without further purification. Optical rotations were measured with a JASCO P-2000 polarimeter, using a sodium lamp (λ = 589 nm) at 22 ºC in 1 cm or 1 dm tubes. NMR experiments were performed at 300 (75.5), 400 (100.6) and 500 (125.7) MHz. 1-D TOCSY as well as 2-D COSY and HMQC experiments were carried out to assist on signal assignment. For ESI mass spectra, 0.1 pm sample concentrations were used, the mobile phase consisting of 50% aq MeCN at 0.1 mL min^{-1}. Thin-layer chromatography was performed on precoated TLC plates, silica gel 30F-245, with visualization by UV light and by carring with 10% H_2SO_4 or 0.2% w/v cerium (IV) suphate-5% ammonium molybdate in 2 M H_2SO_4 or 0.1% ninhydrin in EtOH. Column chromatography was performed on Chromagel (silice 60 AC.C 70-200 μm). Elemental analyses were performed at the Servicio de Microanálisis del Instituto de Investigaciones Químicas de Sevilla, Spain.

For the biological assays, stock solutions of the synthesized compounds in DMSO at 10 mM were prepared. Triton X-100, paraformaldehyde, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), resazurin and phorbol 12-myristate 13-acetate (PMA), were purchased from Sigma-Aldrich (St. Louis, MO). Miltefosine was purchased from Zentaris GmbH (Frankfurt am Main, Germany). DMNPE-luciferin {D-luciferin-1[-(4, 5-dimethoxy-2-nitrophenyl) ethyl ester]}, hygromycin B, and 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) were purchased from Invitrogen (Carlsbad, CA). Kit Luciferase Assay System was purchased from Promega. L-glutamine and penicillin/streptomycin were obtained from Gibco. All chemicals were of the highest quality available. The peracetylated bicyclic nojirimycin derivative (**11**),[22] and the (1*R*)-1-octylamino- (**9**) and (1*R*)-1-octyl-5*N*,6*O*-oxomethylidenenojirimycin (**10**) derivatives were prepared according to previously reported procedures.[19a]

Leishmania culture conditions

Promastigotes of *L. donovani* MHOM/IND/80/Dd8, *L. donovani* MHOM/ET/67/HU3 and the miltefosine-resistant line M-40R[30] used in this study were grown at 28 °C in RPMI 1640-modified medium (Invitrogen) supplemented with 20% heat-inactivated fetal bovine serum (iFBS, Invitrogen).[37] Additionally, *L. donovani* MHOM/IND/80/Dd8 with luciferase gene integrated into the parasite genome (*L. donovani*-LUC) was grown under the same conditions with 100 μg/ml of hygromycin B (unpublished results). Axenic *L. donovani* MHOM/ET/67/HU3 amastigote parasites (provided by Dr. L. Maes form LMPH, University of Antwerp, Belgium) were grown in Schneider medium supplemented with 20% iFBS, pH 5.4 at 37 ºC and 5% CO_2.

Susceptibility analysis in *Leishmania* promastigotes

In drug susceptibility assays, log phase *L. donovani* promastigotes were incubated at 28 °C for 72 h in the presence of increasing concentrations of compounds. Cell viability was determined by the MTT colorimetric assay, as described previously.[38] Miltefosine was used as standard antileishmanial agents.

Susceptibility analysis in *Leishmania* axenic amastigotes

Axenic amastigotes of *L. donovani* (10^6 cell/ml) in a 96-well plate were incubated with increasing concentrations of compounds for 72 h at 37 °C, followed by a resazurin-based assay.[39] Briefly, 40 µl of resazurin (0.02% in milliQ water) were added to each well, incubated for 24 h at 37 ºC and fluorescence was detected at 550-590 nm.

Cell lines culture and determination of cellular toxicity

Human myelomonocytic cell line THP-1 were grown at 37 ºC and 5% CO_2 in RPMI-1640 supplemented with 10% iFBS, 2 mM glutamate, 100 U/mL penicillin and 100 µg/mL streptomycin. 3 x 10^4 cells/well in 96-well plates were differentiated to macrophages with 20 ng/mL of PMA treatment for 48 h followed by 24 h of culture in fresh medium.[40] MRC-5 cells, a SV-40 transformed human fetal lung fibroblast cell line, were maintained at 37 ºC and 5% CO_2 in DMEM supplemented with 10% iFBS, 100 U/mL penicillin and 100 µg/mL streptomycin. Cells were harvested by treatment with 0.05% (w/v) trypsin plus 0.48 mM EDTA for 5 min, diluted to 4 x 10^4 cell/ml in 96-well plates and incubated at 37 ºC and 5% CO_2 before toxicity assay.[41] Cellular toxicity of all compounds was determined using the colorimetric MTT-based assay after incubation at 37 °C for 72 h in the presence of increasing concentrations of compounds.[38] The results are expressed as EC_{50} values, as the concentration of compound that reduce cell growth by 50% versus untreated control cells.

Susceptibility analysis in intracellular *Leishmania* amastigotes

Macrophage-differentiated-THP-1 cells, which are considered a suitable model for human macrophages, were plated at a density of $3x10^4$ or $3x10^5$ macrophages/well in 96-well white polystyrene microplates or 24-well tissue culture chamber slides, respectively, and were infected at a macrophage/parasite ratio of 1:10 with *L. donovani* promastigotes. 24 h after infection at 35 ºC and 5% CO_2, extracellular parasites were removed by washing with serum-free medium. Infected cell cultures were then incubated at different compound concentrations in RPMI 1640 medium plus 10% iFBS at 37 °C with 5% CO_2 for 72 h. To determine the susceptibility of *L. donovani*-LUC amastigotes, infected macrophages maintained in 96-well plates were lysed and the luminescence intensity was measured as indicative of the intracellular parasite growth, using the Luciferase Assay System Kit (Promega, Madison, Wis.) according to the instructions of the supplier. To determine the susceptibility of *L. donovani* HU3 amastigotes, infected macrophages maintained in 24-well plates were fixed for 30 min at 4 ºC with 2.5% paraformaldehyde phosphate-buffered saline (PBS; 1.2 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 130 mM NaCl and 2.6 mM KCl adjusted to pH 7) and permeabilized with 0.1% Triton X-100 in PBS for 30 min. Intracellular parasites and macrophages were detected by nuclear staining with ProLong® Gold antifade reagent plus DAPI (Invitrogen). The percentage of infection and the mean number of amastigotes in the infected macrophages were determined in 200 macrophages/well.

Drug interaction analysis

To analyse the combination of miltefosine with the most active compound (**4**), intracellular amastigotes of *L. donovani* were treated with increasing concentrations of the compound at fixed concentrations of miltefosine (0.1, 0.2 and 0.3 µM) and cell viability was evaluated by luciferase assay as described above. A classical isobologram was constructed by plotting two drugs concentrations on the x-axis and y-axis respectively in a two-coordinate plot.[42] The line connecting the concentration of both drugs required to produce a certain dose-response (*e.g.* EC_{50}) is the line of additivity. The concentrations of the two drugs used in combination are place in the same plot, indicating synergy, additivity or antagonism when they are located below, on or above that line, respectively. The combination index (CI) was used to express synergism (CI < 1), additivity (CI = 1) or antagonism (CI > 1) and was calculated according to the classic isobologram equation.[30]

Statistical analysis

All assays were performed in triplicates. Data are presented as the mean ± SD for three independent experiments. Statistical significance was calculated using Student's *t*-test. Differences were considered significant at a level of $p<0.01$.

General procedure for the preparation of pseudothioglycoside sp^2-iminosugar derivatives. To a stirred solution of **11** (404 mg, 1.08 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 ºC, $BF_3.Et_2O$ (0.48 mL, 3.78 mmol, 3.5 equiv.) and the corresponding *n*-alkylthiol (2.27 mmol, 2.1 equiv.) were dropwise added under N_2 atmosphere. The mixture was stirred for 60 min (TLC monitoring), diluted with CH_2Cl_2 (80 mL) and washed with water (15 mL), aq $NaHCO_3$ (15 mL) and water (15 mL), dried (Na_2SO_4) and concentrated to afford the corresponding per-*O*-acetylated thioglycosides **12**/**12β** and **13**/**13β** (α:β ratio 20:1; H-1 integration). The pure anomers were obtained after separation by column chromatography (1:3 EtOAc-cyclohexane). Conventional de-*O*-acetylation of the major α-anomers **12** and **13** with NaOMe in MeOH and subsequent column chromatography (20:1 → 9:1 DCM-MeOH) of the crude product afforded the target fully unprotected thioglycosides **1** and **2** in 92% and quantitative yield, respectively.

The octyl pseudothioglycosides **12**/**12β** and **1** exhibited identical physicochemical data to those already reported in a preliminary communication.[19a] The corresponding data for the α-configured thiododecyl analogues **13** and **2** are listed hereinafter, whereas data for the minor β-diastereomers **13β** and **2β** are collected in the Supplementary information.

(1*R*)-2,3,4-Tri-*O*-acetyl-1-dodecylthio-5*N*,6*O*-oxomethylidenenojirimycin (13). Yield: 527 mg (95%). R_f 0.69 (1:1 EOAc-cyclohexane). $[\alpha]_D$ +72.5 (*c* 1.0 in DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.66 (d, 1 H, $J_{1,2}$ = 5.8 Hz, H-1),

5.41 (t, 1 H, $J_{2,3} = J_{3,4} = 9.8$ Hz, H-3), 4.95 (dd, 1 H, H-2), 4.92 (t, 1 H, $J_{4,5} = 9.5$ Hz, H-4), 4.44 (dd, 1 H, $J_{6a,6b} = 9.0$ Hz, $J_{5,6a} = 8.5$ Hz, H-6a), 4.27 (dd, 1 H, $J_{5,6b} = 6.6$ Hz, H-6b), 4.15 (ddd, 1 H, H-5), 2.60 (m, 1 H, SCH_2), 2.47 (m, 1 H, SCH_2), 2.08 (s, 3 H, MeCO), 2.05 (s, 3 H, MeCO), 2.02 (s, 3 H, MeCO), 1.63-1.51 (m, 2 H, SCH_2CH_2), 1.36-1.24 (m, 18 H, CH_2), 0.89 (t, 3 H, $^3J_{H,H} = 7.0$ Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.1-169.6 (CO ester), 155.5 (CO carbamate), 72.8 (C-4), 70.4 (C-2), 70.0 (C-3), 66.4 (C-6), 57.9 (C-1), 51.4 (C-5), 32.0-22.8 (CH_2), 20.7-20.6 (*Me*CO), 14.2 (CH_3). ESIMS: *m/z* 538.2 [M + Na]$^+$. Anal. Calcd for $C_{25}H_{41}NO_8S$: C, 58.23; H, 8.01; N, 2.72; S, 6.22. Found: C, 58.31; H, 8.121; N, 2.65; S, 6.17.

(1*R*)-1-Dodecylthio-5*N*,6*O*-oxomethylidenenojirimycin (2): R_f 0.47 (9:1 DCM-MeOH). $[\alpha]_D$ +94.8 (*c* 1.0 in MeOH) ^{1}H NMR (500 MHz, CD_3OD) δ 5.25 (d, 1 H, $J_{1,2} = 5.6$ Hz, H-1), 4.55 (t, 1 H, $J_{6a,6b} = J_{5,6a} = 8.7$ Hz, H-6a), 4.27 (dd, 1 H, $J_{5,6b} = 5.8$ Hz, H-6b), 3.92 (ddd, 1 H, $J_{4,5} = 9.5$ Hz, H-5), 3.65 (dd, 1 H, $J_{2,3} = 9.6$ Hz, H-2), 3.53 (t, 1 H, $J_{3,4} = 9.2$ Hz, H-3), 3.32 (dd, 1 H, H-4), 2.61-2.56 (m, 1 H, SCH_2), 2.53-2.48 (m, 1 H, SCH_2), 1.68-1.54 (m, 2 H, SCH_2CH_2), 1.43-1.39 (m, 2 H, CH_2), 1.36-1.29 (m, 16 H, CH_2), 0.90 (t, 3 H, $^3J_{H,H} = 7.0$ Hz, CH_3). ^{13}C NMR (125.7 MHz, CD_3OD) δ 158.4 (CO), 75.7 (C-4), 75.2 (C-3), 72.5 (C-2), 68.2 (C-6), 62.5 (C-1), 54.5 (C-5), 33.0-23.7 (CH_2), 14.4 (CH_3). ESIMS: *m/z* 388.0 [M - H]$^-$. Anal. Calcd for $C_{19}H_{35}NO_5S$: C, 58.58; H, 9.06; N, 3.60; S, 8.23. Found: C, 58.38; H, 8.98; N, 3.88; S, 8.57.

General procedure for the preparation of sulfoxide derivatives from sufide precursors. To a solution of (1*R*)-1-octyl(dodecyl)thio-5*N*,6*O*-oxomethylidenenojirimycin (**12** or **13**) (0.23 mmol) in DCM (6 mL), MCPBA (70%, 41 mg, 0.23 mmol) was added at 0 ºC. The reaction mixture was stirred for 10 min (TLC monitoring), diluted with DCM (50 mL), washed with aqueous $NaHCO_3$ (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The resulting crude was purified by column chromatography to give the corresponding octyl(dodecyl)sulfoxide.

(1*R*)-1-Octylsulfinyl-2,3,4-tri-*O*-acetyl-5*N*,6*O*-oxomethylidenenojirimycin (14). Column chromatography (1:1 EtOAc-cyclohexane). Yield: 33 mg (30%). R_f 0.55 (3:2 EtOAc-cyclohexane). $[\alpha]_D$ +38.6 (*c* 0.8 in DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.85 (t, 1 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), 5.25 (dd, 1 H, $J_{1,2} = 7.1$ Hz, H-2), 4.98 (t, 1 H, $J_{4,5} = 9.0$ Hz, H-4), 4.91 (d, 1 H, H-1), 4.66 (td, 1 H, $J_{5,6a} = 9.0$ Hz, $J_{5,6b} = 5.1$ Hz, H-5), 4.44 (t, 1 H, $J_{6a,6b} = 9.0$ Hz, H-6a), 4.22 (dd, 1 H, H-6b), 2.79 (ddd, 1 H, $^2J_{H,H} = 13.2$ Hz, $^3J_{H,H} = 9.0$ Hz, $^3J_{H,H} = 6.0$ Hz, $SOCH_2$), 2.62 (ddd, 1 H, $SOCH_2$), 2.07-1.97 (3 s, 9 H, MeCO), 1.80-1.10 (m, 12 H, CH_2), 0.81 (t, 3 H, $^3J_{H,H} = 7.0$ Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.1-169.2 (CO ester), 156.6 (CO carbamate), 71.6 (C-4), 69.6 (C-3), 69.4 (C-2), 66.5 (C-6, C-1), 54.5 (C-5), 49.7 ($SOCH_2$), 31.7-22.5 (CH_2), 20.6-20.5 (*Me*CO), 14.0 (CH_3). ESIMS: *m/z* 498.1 [M + Na]$^+$. Anal. Calcd for $C_{21}H_{33}NO_9S$: C 53.04, H 6.99, N 2.95, S 6.74. Found: C 52.78, H 6.67, N 2.63, S 6.53.

(1*R*)-1-Octylsulfinyl-2,3,4-tri-*O*-acetyl-5*N*,6*O*-oxomethylidenenojirimycin (15). Column chromatography (1:1 EtOAc:cyclohexane). Yield: 44 mg (39%). R_f 0.40 (3:2 EtOAc-cyclohexane). $[\alpha]_D$ +33.5 (*c* 1.0 in DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.50 (dd, 1 H, $J_{2,3} = 7.0$ Hz, $J_{3,4} = 5.0$ Hz, H-3), 5.45 (bt, 1 H, H-2), 4.98 (d, 1 H, $J_{1,2} = 5.5$ Hz, H-1), 4.85 (dd, 1 H, $J_{4,5} = 8.3$ Hz, H-4), 4.40 (d, 2 H, $J_{5,6} = 5.5$ Hz, H-6a, H-6b), 3.99 (dt, 1 H, H-5), 2.88 (ddd, 1 H, $^2J_{H,H} = 13.5$ Hz, $^3J_{H,H} = 9.5$ Hz, $^3J_{H,H} = 7.0$ Hz, $SOCH_2$), 2.75 (ddd, 1 H, $SOCH_2$), 2.08-2.00 (3 s, 9 H, MeCO), 1.90-1.66 (m, 2 H, $SO_2CH_2CH_2$), 1.46-1.15 (m, 10 H, CH_2), 0.81 (t, 3 H, $^3J_{H,H} = 7.0$ Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 169.8-168.5 (CO ester), 156.3 (CO carbamate), 72.2 (C-4), 68.8 (C-3), 67.4 (C-1), 67.3 (C-6), 67.1 (C-2), 54.4 (C-5), 50.5 (SO_2CH_2), 31.7-22.5 (CH_2), 20.5-20.4 (*Me*CO), 14.0 (CH_3). ESIMS: *m/z* 498.1 [M + Na]$^+$. Anal. Calcd for $C_{21}H_{33}NO_9S$: C 53.04, H 6.99, N 2.95, S 6.74. Found: C 53.11, H 7.08, N 2.79, S 6.52.

(1*R*)-1-Octylsulfinyl-5*N*,6*O*-oxomethylidenenojirimycin (3). Compound **3** was obtained by conventional de-*O*-acetylation of **14** (29 mg, 0.06 mmol). Yield: 18 mg (85%). R_f 0.78 (1:5 MeOH-EtOAc). $[\alpha]_D$ +63.5 (*c* 1.0 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ 4.62 (t, 1 H, $J_{5,6a} = J_{6a,6b} = 9.0$ Hz, H-6a), 4.28 (dd, 1 H, $J_{5,6b} = 6.4$ Hz, H-6b), 4.06 (ddd, 1 H, $J_{4,5} = 9.5$ Hz, H-5), 3.94 (dd, 1 H, $J_{2,3} = 9.5$ Hz, $J_{1,2} = 6.6$ Hz, H-2), 3.82 (t, 1 H, $J_{3,4} = 9.5$ Hz, H-3), 3.35 (t, 1 H, H-4), 2.97 (ddd, 1 H, $^2J_{H,H} = 13.0$ Hz, $^3J_{H,H} = 9.0$ Hz, $^3J_{H,H} = 7.0$ Hz, SO_2CH_2), 2.90 (ddd, 1 H, SO_2CH_2), 1.87-1.72 (m, 2 H, $SO_2CH_2CH_2$), 1.55-1.27 (m, 10 H, CH_2), 0.91 (t, 3 H, $^3J_{H,H} = 7.0$ Hz, CH_3). ^{13}C NMR (125.7 MHz, CD_3OD) δ 159.4 (CO), 75.7 (C-3), 74.8 (C-4), 72.5 (C-1), 71.6 (C-2), 68.7 (C-6), 57.5 (C-5), 52.3 ($SOCH_2$), 32.9-23.7 (CH_2), 14.4 (CH_3). ESIMS: *m/z* 372.0 [M + Na]$^+$. Anal. Calcd for $C_{15}H_{27}NO_6S$ C 51.56, H 7.79, N 4.01, S 9.18. Found: C 51.33, H 7.54, N 3.79, S 8.85.

(1*R*)-1-Octylsulfinyl-5*N*,6*O*-oxomethylidenenojirimycin (5). Compound **5** was obtained by conventional de-*O*-acetylation of **15** (33 mg, 0.07 mmol). Yield: 21 mg (87%). R_f 0.67 (1:5 MeOH-EtOAc). $[\alpha]_D$ +53.6 (*c* 0.5 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ 4.89 (d, 1 H, $J_{1,2} = 5.9$ Hz, H-1), 4.58 (t, 1 H, $J_{5,6a} = J_{6a,6b} = 9.0$ Hz, H-6a), 4.30 (dd, 1 H, $J_{5,6b} = 5.0$ Hz, H-6b), 4.07 (dd, 1 H, $J_{2,3} = 9.0$ Hz, H-2), 3.94 (t, 1 H, $J_{3,4} = 9.0$ Hz, H-3), 3.91 (ddd, 1 H, $J_{4,5} = 8.0$ Hz, H-5), 3.39 (dd, 1 H, H-4), 3.14 (ddd, 1 H, $^2J_{H,H} = 13.2$ Hz, $^3J_{H,H} = 9.5$ Hz, $^3J_{H,H} = 7.0$ Hz, $SOCH_2$), 3.00 (ddd, 1 H, $SOCH_2$), 1.90-1.72 (m, 2 H, $SO_2CH_2CH_2$), 1.58-1.27 (m, 10 H, CH_2), 0.91 (t, 3 H, $^3J_{H,H} = 7.0$ Hz, CH_3). ^{13}C NMR (125.7 MHz, CD_3OD) δ 158.6 (CO), 75.4 (C-3), 75.1 (C-4), 72.9 (C-2), 72.3 (C-1), 68.6 (C-6), 56.9 (C-5), 50.8 ($SOCH_2$), 32.9-23.7 (CH_2), 14.4 (CH_3). ESIMS: *m/z* 372.0 [M + Na]$^+$. HRFABMS Calcd for $C_{15}H_{27}NO_6SNa$ [M + Na]$^+$ 372.1457, found 372.1452.

(1*R*)-2,3,4-Tri-*O*-acetyl-1-dodecylsulfinyl-5*N*,6*O*-oxomethylidenenojirimycin (16). Column chromatography (1:2 EtOAc-cyclohexane). Yield: 152 mg (30%). R_f 0.38 (1:1 EtOAc-cyclohexane). $[\alpha]_D$ +42.3 (*c* 1.2 in DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.92 (t, 1 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), 5.39 (dd, 1 H, $J_{1,2} = 7.1$ Hz, H-2), 5.10 (t, 1 H, $J_{4,5} = 9.7$ Hz, H-4), 4.99 (d, 1 H, H-1), 4.71 (ddd, 1 H, $J_{5,6a} = 9.0$ Hz, $J_{5,6b} = 5.0$ Hz, H-5), 4.50 (t, 1 H, $J_{6a,6b} = 9.0$ Hz, H-6a), 4.29 (dd, 1 H, H-6b), 2.85 (ddd, 1 H, $^2J_{H,H} = 13.0$ Hz, $^3J_{H,H} = 9.0$ Hz, $^3J_{H,H} = 6.0$ Hz, $SOCH_2$), 2.69 (ddd, 1 H, $SOCH_2$), 2.14-2.04 (3 s, 9 H, MeCO), 1.86-1.67 (m, 2 H, $SOCH_2CH_2$), 1.50-1.20 (m, 18 H, CH_2), 0.88 (t, 3 H, $^3J_{H,H} = 7.0$ Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.1-169.2 (CO ester), 156.7 (CO carbamate), 71.6 (C-4), 69.6 (C-3), 69.2 (C-2), 66.5 (C-6, C-1), 54.5 (C-5), 49.5 ($SOCH_2$), 31.8-22.6 (CH_2), 20.5 (MeCO), 14.1 (CH_3).

ESIMS: *m/z* 554.4 [M + Na]$^+$. Anal. Calcd for $C_{25}H_{41}NO_9S$: C 56.48, H 7.77, N 2.63, S 6.03. Found: C 56.14, H 7.55, N 2.58, S 6.38.

(1*R*)-2,3,4-Tri-*O*-acetyl-1-dodecylsulfinyl-5*N*,6*O*-oxomethylidenenojirimycin (17). Column chromatography (1:2 EtOAc-cyclohexane). Yield: 225 mg (44%). R_f 0.33 (1:1 EtOAc-cyclohexane). $[\alpha]_D$ +25.1 (*c* 1.1 in DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.54 (dd, 1 H, $J_{2,3}$ = 7.6 Hz, $J_{3,4}$ = 5.7 Hz, H-3), 5.46 (dd, 1 H, $J_{1,2}$ = 5.7 Hz, H-2), 5.00 (d, 1 H, H-1), 4.93 (dd, 1 H, $J_{4,5}$ = 8.6 Hz, H-4), 4.44-4.37 (m, 2 H, H-6a, H-6b), 4.05-3.99 (m, 1 H, H-5), 2.90 (ddd, 1 H, $^2J_{H,H}$ = 12.0 Hz, $^3J_{H,H}$ = 9.0 Hz, $^3J_{H,H}$ = 5.1 Hz, $SOCH_2$), 2.76 (ddd, 1 H, $SOCH_2$), 2.10-1.98 (3 s, 9 H, MeCO), 1.85-1.65 (m, 2 H, $SOCH_2CH_2$), 1.46-1.14 (m, 18 H, CH_2), 0.81 (t, 3 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 168.9-168.0 (CO ester), 155.3 (CO carbamate), 71.1 (C-4), 67.9 (C-3), 66.4 (C-1, C-2), 66.2 (C-6), 53.2 (C-5), 49.4 ($SOCH_2$), 30.9-21.6 (CH_2), 19.5 (*Me*CO), 13.1 (CH_3). ESIMS: *m/z* 554.4 [M + Na]$^+$. Anal. Calcd for $C_{25}H_{41}NO_9S$: C 56.48, H 7.77, N 2.63, S 6.03. Found: C 56.55, H 7.81, N 2.44, S 5.79.

(1*R*)-1-Dodecylsulfinyl-5*N*,6*O*-oxomethylidenenojirimycin (4). Compound **4** was obtained by conventional de-*O*-acetylation of **16** (32 mg, 0.06 mmol). Yield: 23 mg (93%). R_f 0.63 (9:1 EtOAc-MeOH). $[\alpha]_D$ +58.1 (*c* 1.3 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ 4.62 (t, 1 H, $J_{5,6a}$ = $J_{6a,6b}$ = 8.7 Hz, H-6a), 4.28 (dd, 1 H, $J_{5,6b}$ = 6.4 Hz, H-6b), 4.05 (ddd, 1 H, $J_{4,5}$ = 9.5 Hz, H-5), 3.94 (dd, 1 H, $J_{2,3}$ = 9.5 Hz, $J_{1,2}$ = 6.6 Hz, H-2), 3.81 (t, 1 H, $J_{3,4}$ = 9.5 Hz, H-3), 3.35 (t, 1 H, H-4), 2.97 (ddd, 1 H, $^2J_{H,H}$ = 13.0 Hz, $^3J_{H,H}$ = 9.0 Hz, $^3J_{H,H}$ = 7.3 Hz, $SOCH_2$), 2.90 (ddd, 1 H, $SOCH_2$), 1.86-1.74 (m, 2 H, $SO_2CH_2CH_2$), 1.56-1.24 (m, 18 H, CH_2), 0.90 (t, 3 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (75.5 MHz, CD_3OD) δ 159.4 (CO), 75.6 (C-3), 74.8 (C-4), 72.5 (C-1), 71.6 (C-2), 68.7 (C-6), 57.4 (C-5), 52.3 ($SOCH_2$), 33.1-23.7 (CH_2), 14.4 (CH_3). ESIMS: *m/z* 428.4 [M + Na]$^+$. Anal. Calcd for $C_{19}H_{35}NO_6S$: C 56.27, H 8.70, N 3.45, S 7.91. Found: C 55.92, H 8.63, N 3.17, S 7.54.

(1*R*)-1-Dodecylsulfinyl-5*N*,6*O*-oxomethylidenenojirimycin (6). Compound **6** was obtained by conventional de-*O*-acetylation of **17** (52 mg, 0.10 mmol). Yield: 35 mg (88%). R_f 0.63 (9:1 EtOAc-MeOH). $[\alpha]_D$ +60.5 (*c* 0.8 in DMSO). ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.57 (d, 1 H, $J_{1,2}$ = 5.7 Hz, H-1), 4.47 (t, 1 H, $J_{5,6a}$ = $J_{6a,6b}$ = 8.3 Hz, H-6a), 4.15 (dd, 1 H, $J_{5,6b}$ = 4.7 Hz, H-6b), 3.91 (dd, 1 H, $J_{2,3}$ = 8.4 Hz, H-2), 3.76 (ddd, 1 H, $J_{4,5}$ = 9.5 Hz, H-5), 3.73 (bt, 1 H, $J_{3,4}$ = 7.5 Hz, H-3), 3.24 (dd, 1 H, H-4), 2.96-2.84 (m, 2 H, $SOCH_2$), 1.73-1.57 (m, 2 H, $SOCH_2CH_2$), 1.45-1.19 (m, 10 H, CH_2), 0.85 (t, 3 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6) δ 156.3 (CO), 73.5 (C-3), 73.3 (C-4), 71.0 (C-1), 70.9 (C-2), 66.7 (C-6), 55.2 (C-5), 48.7 ($SOCH_2$), 31.3-22.1 (CH_2), 13.9 (CH_3). ESIMS: *m/z* 428.4 [M + Na]$^+$. Anal. Calcd for $C_{19}H_{35}NO_6S$: C 56.27, H 8.70, N 3.45, S 7.91. Found: C 56.14, H 8.67, N 3.21, S 7.60.

General procedure for the preparation of sulfone derivatives from sulfide precursors. To a solution of (1*R*)-2,3,4-tri-*O*-acetyl-1-octyl(dodecyl)thio-5*N*,6*O*-oxomethylidenenojirimycin (**12** or **13**) (0.11 mmol) in DCM (3 mL), 70% MCPBA (38 mg, 0.22 mmol) was added at 0 ºC. The reaction mixture was stirred for 10 min, diluted with DCM (50 mL), washed with aqueous $NaHCO_3$ (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The resulting crude was purified by column chromatography to yield the corresponding sulfones **16** and **17**.

(1*R*)-2,3,4-Tri-*O*-acetyl-1-octylsulfonyl-5*N*,6*O*-oxomethylidenenojirimycin (18). Column chromatography (1:2 EtOAc-cyclohexane). Yield: 48 mg (90%). R_f 0.56 (1:1 EtOAc-cyclohexane). $[\alpha]_D$ +26.6 (*c* 0.8 in DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.83 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3), 5.36 (d, 1 H, $J_{1,2}$ = 6.7 Hz, H-1), 5.23 (dd, 1 H, H-2), 4.94 (t, 1 H, $J_{4,5}$ = 9.0 Hz, H-4), 4.50-4.43 (m, 1 H, H-6a), 4.36-4.29 (m, 2 H, H-6b, H-5), 3.02-2.89 (m, 2 H, SO_2CH_2), 2.06-1.98 (3 s, 9 H, MeCO), 1.86-1.67 (m, 2 H, $SO_2CH_2CH_2$), 1.38-1.15 (m, 10 H, CH_2), 0.81 (t, 3 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.1-169.1 (CO ester), 155.5 (CO carbamate), 71.8 (C-4), 68.9 (C-3), 68.1 (C-2), 66.8 (C-6), 65.6 (C-1), 52.9 (SO_2CH_2), 52.7 (C-5), 31.6-21.1 (CH_2), 20.6-20.5 (*Me*CO), 14.0 (CH_3). ESIMS: *m/z* 514.1 [M + Na]$^+$. Anal. Calcd for $C_{21}H_{33}NO_{10}S$: C 51.31, H 6.77, N 2.85, S 6.52. Found: C 51.23, H 6.64, N 2.71, S 6.49.

(1*R*)-1-Octylsulfonyl-5*N*,6*O*-oxomethylidenenojirimycin (7). Compound **7** was obtained by conventional de-*O*-acetylation of **18** (37 mg, 0.07 mmol) followed by purification by column chromatography (4:1 EtOAc-cyclohexane). Yield: 23 mg (84%). R_f 0.31 (EtOAc). $[\alpha]_D$ +30.1 (*c* 0.6 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ 5.12 (d, 1 H, $J_{1,2}$ = 6.5 Hz, H-1), 4.63 (t, 1 H, $J_{5,6a}$ = $J_{6a,6b}$ = 8.8 Hz, H-6a), 4.28 (dd, 1 H, $J_{5,6b}$ = 6.2 Hz, H-6b), 4.14 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 4.03 (td, 1 H, $J_{4,5}$ = 9.5 Hz, H-5), 3.82 (dd, 1 H, H-2), 3.40-3.32 (m, 3 H, H-4, SO_2CH_2), 1.85 (quint., 2 H, $J_{H,H}$ = 7.5 Hz, $SO_2CH_2CH_2$), 1.50-1.25 (m, 10 H, CH_2), 0.91 (t, 3 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (125.7 MHz, CD_3OD) δ 158.4 (CO), 75.4 (C-4), 74.1 (C-3), 72.0 (C-1), 71.9 (C-2), 68.7 (C-6), 56.5 (C-5), 56.3 (SO_2CH_2), 32.9-22.5 (CH_2), 14.4 (CH_3). ESIMS: *m/z* 388.1 [M + Na]$^+$. Anal. Calcd for $C_{15}H_{27}NO_7S$: C 49.30, H 7.45, N 3.83, S 8.77. Found: C 49.04, H 7.20, N 3.56, S 8.44.

(1*R*)-2,3,4-Tri-*O*-acetyl-1-dodecylsulfonyl-5*N*,6*O*-oxomethylidenenojirimycin (19). Following the procedure described above for preparation of **18**, compound **19** was obtained. Column chromatography (1:4 EtOAc-cyclohexane). Yield: 60 mg (78%). R_f 0.50 (1:1 EtOAc-cyclohexane). $[\alpha]_D$ +32.6 (*c* 1.0 in DCM). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.83 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3), 5.37 (d, 1 H, $J_{1,2}$ = 6.7 Hz, H-1), 5.23 (dd, 1 H, H-2), 4.94 (t, 1 H, $J_{4,5}$ = 9.0 Hz, H-4), 4.44-4.50 (m, 1 H, H-6a), 4.37-4.29 (m, 2 H, H-6b, H-5), 2.98 (ddd, 1 H, $^2J_{H,H}$ = 13.8 Hz, $^3J_{H,H}$ = 10.2 Hz, $^3J_{H,H}$ = 5.7 Hz, SO_2CH_2), 2.93 (ddd, 1 H, SO_2CH_2), 2.07-1.98 (3 s, 9 H, MeCO), 1.87-1.67 (m, 2 H, $SO_2CH_2CH_2$), 1.38-1.14 (m, 18 H, CH_2), 0.81 (t, 3 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.2-169.1 (CO ester), 155.5 (CO carbamate), 71.8 (C-4), 68.9 (C-3), 68.1 (C-2), 66.8 (C-6), 65.6 (C-1), 52.9 (SO_2CH_2), 52.7 (C-5), 31.9-21.1 (CH_2), 20.6-20.5 (*Me*CO), 14.1 (CH_3). ESIMS: *m/z* 570.4 [M + Na]$^+$. HRFABMS Calcd for $C_{25}H_{41}NO_{10}SNa$ [M + Na]$^+$ 570.2349, found 570.2369.

(1*R*)-1-Dodecylsulfonyl-5*N*,6*O*-oxomethylidenenojirimycin (8). Compound **8** was obtained by conventional de-*O*-acetylation of **19** (33 mg, 0.06 mmol) followed by purification by column chromatography (15:1 EtOAc-MeOH). Yield: 21 mg (83%). R_f 0.56 (9:1 EtOAc-MeOH). $[\alpha]_D$ +13.5 (*c* 1.0 in DMSO). ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.94 (d, 1 H, $J_{1,2}$ = 6.4 Hz, H-1), 4.57 (t, 1 H, $J_{5,6a}$ = $J_{6a,6b}$ = 8.7 Hz, H-6a), 4.15 (dd, 1 H, $J_{5,6b}$ = 6.1 Hz, H-6b), 3.92 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3), 3.84-3.78 (m, 1 H, H-5), 3.77

(dd, 1 H, H-2), 3.40-3.20 (m, 3 H, H-4, SO_2CH_2), 1.75-1.65 (m, 2 H, $SO_2CH_2CH_2$), 1.40-1.20 (m, 18 H, CH_2), 0.86 (t, 3 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6) δ 155.6 (CO), 73.6 (C-4), 71.9 (C-3), 70.6 (C-1), 70.1 (C-2), 66.8 (C-6), 54.7 (C-5), 54.4 (SO_2CH_2), 31.3-23.0 (CH_2), 13.9 (CH_3). ESIMS: *m/z* 444.3 [M + Na]$^+$. Anal. Calcd for $C_{19}H_{35}NO_7S$: C 54.13, H 8.37, N 3.32, S 7.61. Found: C 53.81, H 8.15, N 3.01, S 7.36.

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†Electronic Supplementary Information (ESI) available: General procedure for the glycosidase inhibition assay, Lineweaver-Burk and double reciprocal analysis plots of **2α**, **5**, **7**, **8**, full experimental data for compounds **13β**, **2β** and copies of the ^{1}H and ^{13}C NMR spectra of all new compounds.

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