European Journal of Medicinal Chemistry 255 (2023) 115390

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: www.elsevier.com/locate/ejmech

Research paper

Fluoro-labelled sp^2-iminoglycolipids with immunomodulatory properties

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ARTICLE INFO

Keywords:
Fluorinated glycomimetics
Selectfluor
Immunomodulation
Cancer
Inflammation
p38α MAPK

ABSTRACT

The unique electronic properties of the fluorine atom make its strategic incorporation into a bioactive compound a very useful tool in the design of drugs with optimized pharmacological properties. In the field of the carbohydrates, its selective installation at C2 position has proven particularly interesting, some 2-deoxy-2-fluorosugar derivatives being currently in the market. We have now transferred this feature into immunoregulatory glycolipid mimetics that contain a sp^2-iminosugar moiety, namely sp^2-iminoglycolipids (sp^2-IGLs). The synthesis of two epimeric series of 2-deoxy-2-fluoro-sp^2-IGLs, structurally related to nojirimycin and mannonojirimycin, has been accomplished by sequential Selectfluor-mediated fluorination and thioglycosidation of sp^2-iminoglycals. Exclusively the α-anomer is obtained regardless of the configurational profile of the sp^2-IGL (D-*gluco* or D-*manno*), highlighting the overwhelming anomeric effect in these prototypes. Notably, the combination of a fluorine atom at C2 and an α-oriented sulfonyl dodecyl lipid moiety in compound **11** led to remarkable anti-proliferative properties, featuring similar GI_{50} values than the chemotherapy drug Cisplatin against several tumor cell lines and better selectivity. The biochemical data further evidence a strong reduction of the number of tumor cell colonies and apoptosis induction. Mechanistic investigations revealed that this fluoro-sp^2-IGL induces the non-canonical activation mode of the mitogen-activated protein kinase signaling pathway, causing p38α autoactivation under an inflammatory context.

1. Introduction

The replacement of a hydroxyl group by fluorine or a fluorinated functional group in a bioactive compound is often used in medicinal chemistry to optimize the pharmacological properties because of the unique properties of this atom [1,2]. The strategic location of fluorine motifs can exert beneficial effects on the inherent biological activity of drugs: metabolic/chemical stability, membrane permeability, hydrogen bonding capacity, fluorine-target interactions and enhanced bioavailability [3,4]. Not surprisingly, fluorine-containing molecules are widely distributed within pharmaceuticals. Approximately 20% of commercial drugs against cancer, diabetes, respiratory, infectious, metabolic and cardiovascular diseases are fluorocompounds [5,6]. A number of 2-deoxy-2-fluoro-sugars are also marketed drugs, such as the nucleoside analogues Clevudine (**1** , anti-hepatitis B) [7], Clorafabine (**2** , anti-leukemia) [8] and Sofosbuvir (**3** , anti-hepatitis C) [9] (Fig. 1A). Likewise, some pyranose-based carbohydrate derivatives have displayed better pharmacological profile after fluorine installation at C2 position. This is the case for 2-deoxy-2-fluoro-D-glucose (**4**) [10,11], the fluorinated *C*- and *O*-glycoside analogues of the antidiabetic Dapagliflozin (**5**) [12] and the antitumor Doxorubicin (**6**) [13], respectively (Fig. 1B). The benefit of fluorine chemistry has also been explored on iminosugar-based glycomimetics [14]. Thus, the approved drugs Migalastat (Galafold) for Fabry disease treatment and the antidiabetic Miglitol (Glyset™), have been structurally modified through fluorination reactions to obtain 2-deoxy-2-fluoromigalastat (**7**) [15] and 2-deoxy-2-fluoromiglitol (**8**). The latter showed amplified α-glucosidase inhibition and reduced cytotoxicity as compared with the parent

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https://doi.org/10.1016/j.ejmech.2023.115390
Received 2 March 2023; Received in revised form 14 April 2023; Accepted 17 April 2023
Available online 25 April 2023

iminosugar [16] (Fig. 1C).

Among the battery of synthetic strategies to access 2-deoxy-2-fluoro-sugars [17], the reaction of Selectfluor [18] (1-chloromethyl-4-fluoro-1, 4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) with glycals (1, 2-unsaturated sugars) is probably the most versatile. This reagent allows the regioselective incorporation of fluorine at C2 with the simultaneous functionalization of C1 through the addition of a nucleophile. The fluorination-nucleophilic addition reaction stereoselectivity is governed by factors such as glycal protecting groups, solvents and nature of nucleophiles employed. Yet, the C2-axial and -equatorial diastereomers along with α- and β-anomeric mixtures are usually obtained [19–21]. We hypothesized that this shortcoming could be highly mitigated in the case of sp^2-iminosugars. The presence of a pseudoamide-type nitrogen atom, with a high sp^2-hybridized character, in place of the characteristic endocyclic oxygen of monosaccharides imparts stability to anomerically substituted derivatives [22]. This is drastically different form classical iminosugars, for which the (thio)aminal function is intrinsically unstable. Moreover, the exacerbated anomeric effect in sp^2-iminosugars results in total α-stereocontrol in glycosidation reactions. Notably, a series α-linked *C*-, *N*-, *S*- and *Se*-glycolipid analogues (sp^2-iminoglycolipids; sp^2-IGLs) with immunoregulatory properties have been reported [23–25]. Amongst them, (1*R*)-1-dodecylsulfonyl-5*N*,6*O*-oxomethylidenenojirimycin (DSO_2-ONJ, **9**) (Fig. 1D) stands out. DSO_2-ONJ has shown promise for the treatment of diabetic retinopathy (DR) [26], acute inflammation [27] and tumor proliferation [28], promoting the resolution of inflammatory events. Biochemical and computational data support a mechanism of action implying non-canonical activation of p38α mitogen-activated protein kinase (MAPK) [26]. Indeed, p38α MAPK is a master regulator of inflammation and a highly pursued therapeutic target in diseases such as cancer, diabetes, parasitic infections or neurological disorders, amongst others [29]. The preparation of fluorinated analogues of DSO_2-ONJ is, therefore, very attractive in a medicinal chemistry context. Here we present the stereoselective synthesis of a series of 2-deoxy-2-fluoro-α-sp^2-IGLs (**10** –**17**) and their biological evaluation as anti-proliferative and anti-inflammatory agents. Emphasis is placed in discerning the effects of replacing OH by F at C2 position, the presence or not of an additional lipophilic substituent at C3 and the configurational profile (D-*gluco* or D-*manno*) on the biological activity (Fig. 2). The corresponding reducing 2-deoxy-2-fluoro-glycomimetics (**19** –**22**) and the previously reported fully hydroxylated (1*R*)-5*N*,6*O*-oxomethylidenenojirimycin (ONJ) [22] and (1*R*)-5*N*, 6*O*-oxomethylidene-mannonojirimycin (OMJ) [30], along with the sp^2-IGLs **9** , **23** (ONJ-related) [31] and **24** , **25** (OMJ-related) [27] were also included in the study for structure-activity relationship (SAR) purposes (Fig. 2).

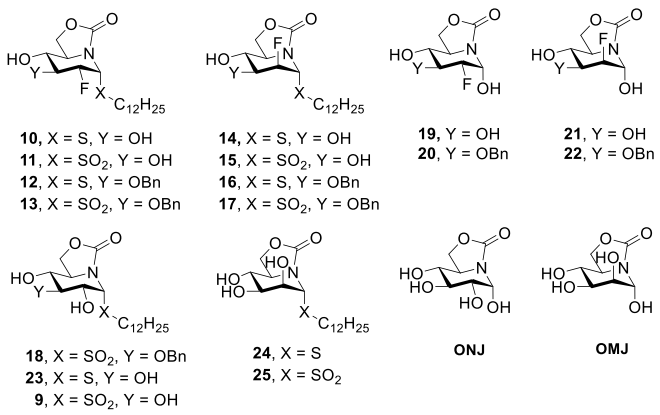


Fig. 2. Chemical structures evaluated in this study.

2. Results

2.1. Design and synthesis

The synthetic strategy to achieve the target 2-deoxy-2-fluoroglycomimetics was initially designed from the di-*O*-acetylated iminoglycal carbamate (**26**) [32]. The regioselective incorporation of the fluorine atom at C2 along with the concurrent functionalization of the pseudoanomeric center was mediated by Selectfluor in a 5:1 mixture of nitromethane-water (Scheme 1). The reducing crude product was straightforward acetylated, yielding a separable mixture of epimers at C2 (F_{eq}/F_{ax}) in 0.7/1 ratio (^{1}H NMR). Column chromatography afforded 32% of D-*gluco* (**27**) and 48% of D-*manno* (**28**) fluorinated products in pure form. The stereochemistry at C2 was inferred from the vicinal $J_{2,3}$ coupling constant values, ~10 Hz for **27** and < 3 Hz for **28** . Considering that the ensemble of vicinal proton-proton coupling constants are consistent with the $^4C_1$ chair conformation for the piperidine ring, such $J_{2,3}$ values are characteristic of *anti* (F_{eq}) and *gauche* dispositions (F_{ax}), respectively. The α-anomer was exclusively obtained in both cases ($J_{1,2}$ = 3.0–3.6 Hz), underlining the overwhelming anomeric effect in sp^2-iminosugars. Decoupled ^{19}F NMR (282 MHz, $CDCl_3$) spectra showed a single signal for compounds **27** and **28** , corresponding to the incorporation of the fluorine atom (−200.4 and −201.5 ppm, respectively).

A detailed study of the fluorination reaction stereoselectivity as a function of the glycal protecting groups was next conducted, including ester (benzoyl **30** and pivaloyl **31**), ether (benzyl **32** and the cyclic *o*-xylylenyl **33**) as well as a combination of ester and ether substituents (acetyl and benzyl **34** ; Schemes 2 and 3).

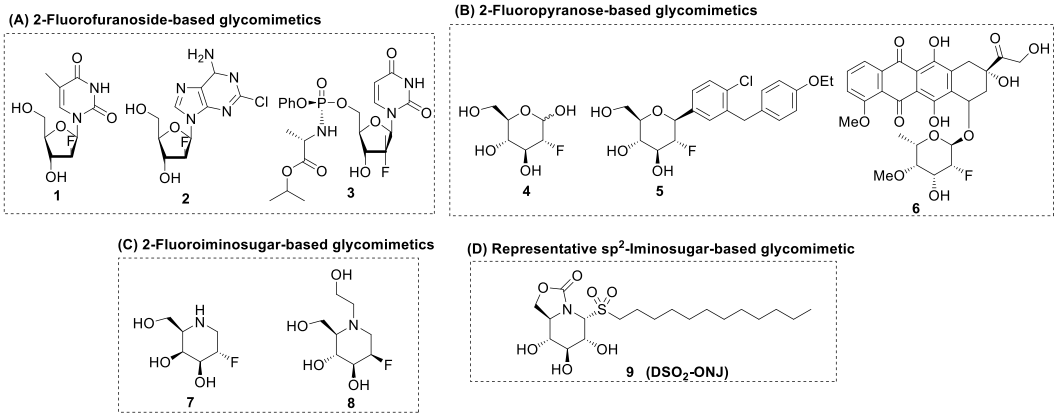
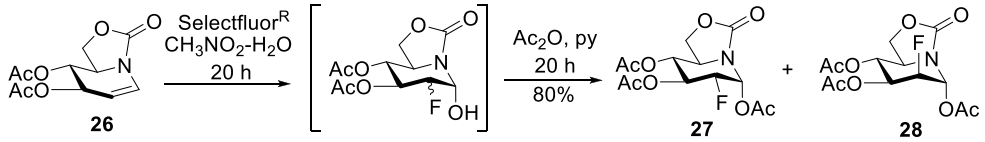
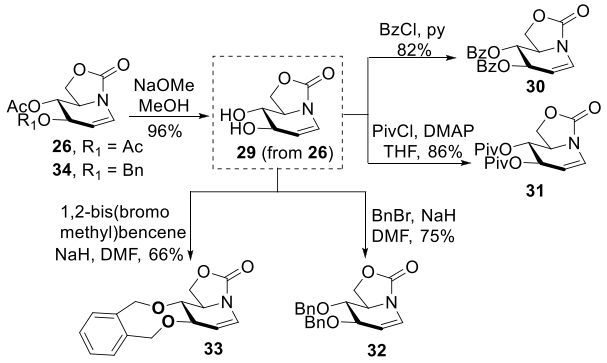


Fig. 1. Chemical structures of 2-deoxy-2-fluoro-glycomimetics: (**A**) *N*-furanosides (**1** –**3**) incorporated in the pharmaceutical market. (**B**) 2-Deoxy-2-fluoro-D-glucose (**4**) and 2-fluorinated glycomimetics of Dapagliflozin and Doxorubicin (**5** , **6**). (**C**) Piperidine-based fluorinated iminosugars (**7** , **8**). (**D**) Structure of **9** (DSO_2-ONJ) as a leading candidate of the sp^2-IGLs family.



Scheme 1. Selectfluor-mediated fluorination reaction of the sp^2-iminoglycal carbamate (**26**).



Scheme 2. Synthesis of sp^2-iminoglycal analogues (**30** –**33**) bearing different protecting groups.

De-*O*-acetylation of **26** under Zemplén conditions [33] afforded the unprotected sp^2-iminoglycal **29** [34] as pivotal intermediate for the synthesis of the protected glycals **30** , **31** , **32** [34] and **33** . Benzoyl chloride and pivaloyl chloride, were used for the synthesis of **30** and **31** (82% and 86% yield, respectively), and benzyl bromide and 1,2-bis(bromomethyl)benzene for the synthesis of compounds **32** and **33** (75% and 66% yield, respectively; Scheme 2).

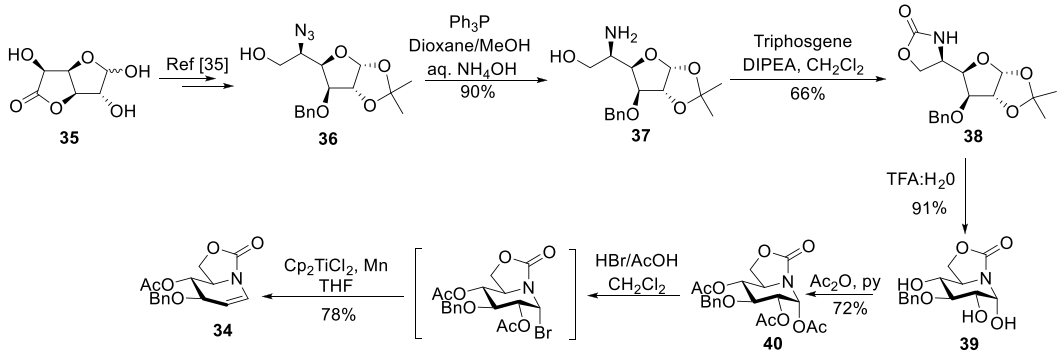
The synthesis of the sp^2-iminoglycal **34** , bearing a benzyl group at O3, started from commercially available D-glucofuranurono-γ-lactone (**35**), which was transformed into azide **36** following reported procedures [35]. Reduction to the corresponding amine group (→ **37**) using Staudinger conditions [36], followed by carbonylation reaction with triphosgene and *N*,*N*-diisopropylethylamine (DIPEA), led to carbamate **38** . Removal of the acetonide group with trifluoroacetic acid-water (9:1) proceeded with concomitant intramolecular cyclization to afford the fused piperidine-carbamate bicyclic system **39** in excellent yield, which was next acetylated to give **40** . Transformation into the corresponding α-pseudoglycosyl bromide and subsequent treatment with titanocene dichloride (Cp_2TiCl_2) and manganese led to **34** (78% yield after purification by column chromatography; Scheme 3).

All the synthesized sp^2-iminoglycals underwent electrophilic fluorination reaction mediated by Selectfluor under the conditions stated in Scheme 1. The ratios of epimeric compounds at C2 (H-1 integration, ^{1}H NMR of the crude product) are collected in Table 1. The α-anomer was exclusively obtained in all cases regardless of the protecting group, greatly simplifying the purification step.

The data evidenced that ester protecting groups favored the formation of the D-*gluco* over D-*manno* fluorinated product, the di-*O*-pivaloylated derivative **31** (Table 1, entry 3) affording slightly better results than the di-*O*-benzoylated derivative **30** (Table 1, entry 2). Nevertheless, compound **30** was selected as the most suitable precursor for the synthesis of the target sp^2-IGLs given the milder deprotection conditions of benzoyl groups *vs* pivaloyl groups, the convenient separation of the epimeric mixture using UV–visible detection (→ **41** and **42**) and the compatibility with thioglycosidation reaction conditions.

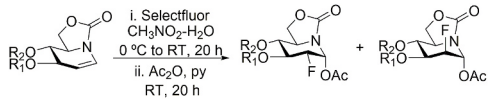
Compounds **41** and **42** were, on the one hand, deacylated (→ **19** and **21**) and, on the other hand, thioglycosylated with dodecane-1-thiol using boron trifluoride etherate (BF_3Et_2O) as promotor (→ **43** and **44**). Debenzoylation afforded the target fully unprotected α-pseudoglycosylsulfides **10** and **14** with excellent yields (Scheme 4). The chemical structure of all new compounds was confirmed by ^{1}H, ^{13}C, ^{19}F NMR, MS and elemental analysis. Notably, the proton-proton $J_{2,3}$ coupling constants allowed unequivocally determining the stereochemistry at C2. In general terms, $J_{2,3}$ values are ranged between 8 and 10 Hz for derivatives in which the fluorine atom is equatorially arranged ($H2_{ax}$-$H3_{ax}$, ONJ-related). Differently, for those compounds in which the fluorine atom is axially oriented, $J_{2,3}$ coupling constants display values ranging between 1.5 and 3.0 Hz ($H2_{eq}$-$H3_{ax}$, OMJ-related).

The pseudo α-glycosylsulfones **45** and **46** were obtained from the corresponding protected α-glycosylsulfides **43** and **44** by using excess of the oxidant reagent *m*-chloroperbenzoic acid (MCPA; Scheme 5). Unexpectedly, deprotection of **45** under basic conditions in methanol did not afford the expected unprotected 2-deoxy-2-fluorosulfonyl derivative **11** . Instead, the reaction proceeded with concomitant nucleophilic displacement of the fluorine atom by a methoxy group with complete inversion of configuration (S_N2), affording the corresponding 2-metoxy-D-mannonojirimycin derivative **47** as the only product. Attempts to conduct the deprotection step under acidic conditions (7% HCl in MeOH) failed to remove the benzoate groups even after prolonged reaction times. As an alternative, the di-*O*-acetylated 2-deoxy-2-fluorosulfonyl glycosides **50** and **51** were employed. The synthesis of **50** from **43** was accomplished in three steps involving Zemplén deprotection (→ **10**), acetylation (→ **48**), and final oxidation of the anomeric sulfur atom. Gratifyingly, deprotection of the acetyl groups of **50** using 7% HCl in



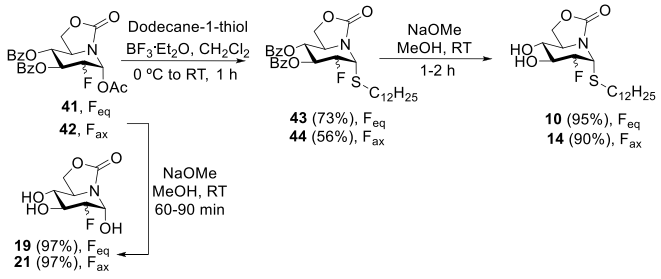
Scheme 3. Synthesis of the sp^2-iminoglycal **34** .

Table 1
Effect of the sp^2-iminoglycal protecting group in the outcome of the electrophilic fluorination reaction mediated by Selectfluor.

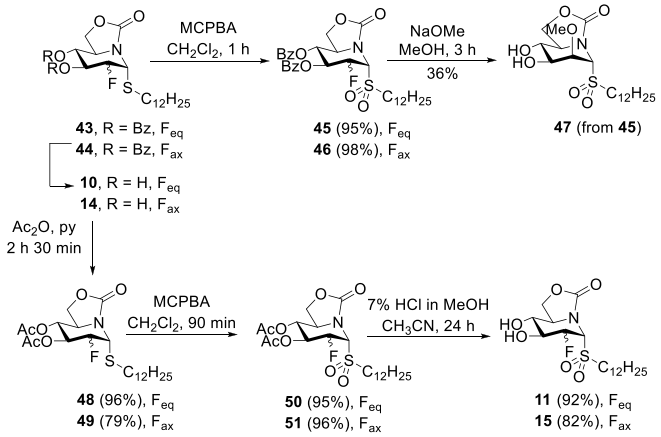


Entry	D-Glycal	Ratio[a] F_{eq}/F_{ax}	Compounds D-*gluco*/D-*manno*	Yield (%)[b] D-*gluco*/D-*manno*	Global yield (%)
1	**26** , $R_1 = R_2 =$ Ac	0.7/1	**27** /**28**	32/48	80
2	**30** , $R_1 = R_2 =$ Bz	1.7/1	**41** /**42**	45/27	72
3	**31** , $R_1 = R_2 =$ Piv	2/1	–	–	–
4	**32** , $R_1 = R_2 =$ Bn	1.4/1	–	–	–
5	**33** , *o*-Xylylene	0.5/1	–	–	–
6	**34** , R_1 = Bn, R_2 = Ac	0.8/1	**52** /**53**	34/43	77

[a] Determined by ^{1}H NMR of the crude product.
[b] Isolated after purification by column chromatography.



Scheme 4. Synthesis of the reducing 2-deoxy-2-fluoro-sp^2-iminosugars (**19** , **21**) and 2-deoxy-2-fluoro pseudo-α-*S*-dodecyl glycosides (**10** , **14**).



Scheme 5. Synthesis of the novel 2-deoxy-2-fluoro α-dodecylsulfonyl derivatives **11** and **15** .

MeOH at room temperature for 24 h afforded the expected α-dodecylsulfonyl nojirimycin derivative **11** in 92% yield. The same synthetic strategy was followed from the benzoylated D-*manno*-fluorinated derivative **44** to achieve **15** (via intermediates **14** , **49** and **51**) in 82% yield (Scheme 5).

For the preparation of the 3-*O*-benzyl-2-deoxy-2-fluoro D-*gluco*- and D-*manno*-configured reducing derivatives **20** and **22** , as well as the sp^2-IGLs **12** , **13** and **16** , **17** , the same synthetic route described in Schemes 4 and 5 was followed using **34** as starting sp^2-iminoglycal, through reaction intermediates **52** , **54** , **56** and **53** , **55** , **57** , respectively (Scheme 6). Compound **18** was also included in our study as a non-fluorinated control. Its synthesis was achieved from precursor **40** by α-thioglycosidation (→ **58**), oxidation (→ **59**) and final de-*O*-acetylation (Scheme 7).

2.2. Anti-proliferative activity of the sp^2-IGLs

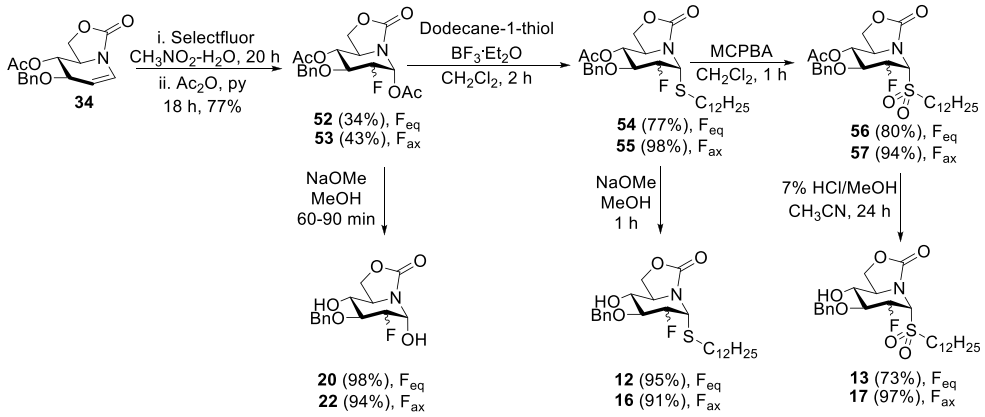
The anti-proliferative potential of compounds **10** –**25** was screened by the sulforhodamine B (SBR) colorimetric assay against the human solid tumor cells lines A549 (non-small cell lung cancer), HBL-100 and T-47D (breast cancer), HeLa (cervical cancer), SW1573 (alveolar cell carcinoma), and WiDr (colon) linages. The 50% growth inhibition values (GI_{50}) are shown in Table 2. It becomes apparent that the presence of the lipid chain is crucial on the anti-proliferative activity of this family of glycomimetics. Thus, the reducing 2-fluorinated nojirimycin (**19** , **20**) and mannonojirimycin derivatives (**21** , **22**) and their non-fluorinated counterparts ONJ and OMJ, respectively, did not show any relevant activity against any of the tumor cell lines evaluated (GI_{50} values above 100 μM). Contrariwise, when the α-oriented dodecyl thioether ($SC_{12}H_{25}$) was incorporated (*gluco* **23** , *manno* **24**), GI_{50} values ranging between 16 and 26 μM were determined; the presence of fluorine instead of hydroxyl at C2 (**10** , **14**), or the presence of the benzyl group at O3 (**12** , **16**) did not lead to any significant difference in the anti-proliferative activity of the α-thioglycosides. However, a significant improvement was observed when the sp^2-IGL combined both the fluorine atom at C2 and the sulfonyl group at the glycosidic linkage, i.e. compounds **11** and **15** . GI_{50} values in the low micromolar range were then achieved for some of the tumor cell lines evaluated. Moreover, a further enhancement was generally observed for the compounds additionally bearing the O3 benzyl group **13** and **17** (GI_{50} values < 10 μM against the whole set of cell lines assayed). The anti-proliferative potency was decreased by an order of magnitude when the fluorine atom was replaced by a methoxyl group (**47**) or a hydroxyl group either equatorially or axially oriented (**9** , **25**), underlining the positive effect of the fluorine atom at C2 in the α-dodecylsulfone sp^2-IGL series.

2.2.1. Selectivity of (1R)-2-deoxy-1-S-dodecyl-2-fluoro-5N,6O-oxomethylidene-1-sulfonylnojirimycin (11)

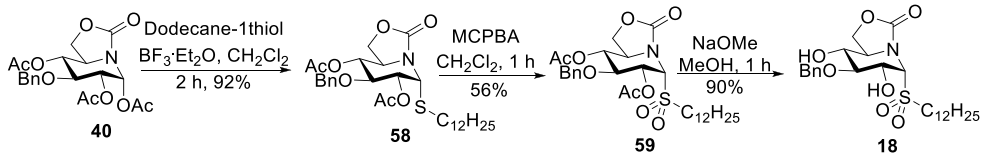
To get a deeper insight on the mechanisms at play, the *in vitro* anti-proliferative activity of the 2-fluorinated nojirimycin derivative **11** was studied using the National Cancer Institute protocol with slight modifications [37]. Besides tumor cells (Table 2) and to determine the selectivity, this compound was tested against the non-tumor BJ-hTERT (human immortalized fibroblast) cell line, reaching a GI_{50} value of 32 ± 5 μM. The results (Table 2 and Fig. 3) indicated that compound **11** showed 1.6-to-7-fold higher selectivity toward the cancer cells used. For comparison, the widely used cancer chemotherapeutic cisplatin is even more cytotoxic in the non-tumor cells BJ-hTERT (GI_{50} = 14 ± 2 μM) than in some of the solid tumor cells (GI_{50} values in Table 2 and Fig. 3).

2.2.2. P-glycoprotein (P-gp) does not affect the activity of the fluorinated sp^2-IGL 11

P-gp is a transmembrane glycoprotein that extrudes cytotoxic drugs from cells. The overexpression of P-gp confers cells resistance to the drugs. To test if this compound could suffer P-gp extrusion, a cell-based assay was used. Thus, the GI_{50} values after 48 h of exposure in wild-type and P-gp-overexpressing SW1573 cells, and in the presence or absence of the P-gp transport inhibitor verapamil (at a fixed concentration of 10 μM) were determined. The standard microtubule-interacting drug paclitaxel (PTX) was the positive control. The results are shown in Table 3. For a better comparison of the data, the resistance factor (R_f) for a given compound is defined as the ratio of GI_{50} values against the P-gp-overexpressing and the wild-type cell lines, respectively. Overall, the



Scheme 6. Synthesis of (1*R*)-3-*O*-benzyl-2-deoxy-2-fluorosugars (**20** , **22**) and (1*R*)-3-*O*-benzyl-2-deoxy-2-fluoro glycosides D-*gluco* (**12** , **13**) and D-*manno* (**16** , **17**) configured.



Scheme 7. Synthesis of (1*R*)-3-*O*-benzyl-1-*S*-dodecyl-5*N*,6*O*-oxomethylidene-1-sulfonylnojirimycin (**18**).

results indicate that, sharply differently from PTX, the activity of the sp^2-IGL **11** is not affected by the overexpression of P-gp.

2.2.3. (1R)-2-Deoxy-1-S-dodecyl-2-fluoro-5N,6O-oxomethylidene-1-sulfonylnojirimycin (11) reduces the number of cell colonies in a concentration-dependent manner

To ponder the long-term effects of compound **11** , the colony formation assay was carried out (Fig. 4). As a rule of thumb, HeLa and SW1573 cells were exposed to two drug doses, namely the high dose, defined as the GI_{50} value (Table 2), and the low dose, fixed to one third of GI_{50}. After seven days of treatment, the ability to form colonies decreased significantly in both cell lines. Interestingly, compound **11** showed more potency in lung SW1573 tumor cells, reducing not only the number of colonies but also their size, even at the low dose.

2.2.4. (1R)-2-Deoxy-1-S-dodecyl-2-fluoro-5N,6O-oxomethylidene-1-sulfonylnojirimycin (11) produces membrane blebbing and cell shrinkage, morphological clues of apoptosis induction

For a deeper phenotypical characterization of the mode of action of this 2-fluorinated-sp^2-IGL, both HeLa and SW1573 cells were exposed to 20 μM of compound **11** . The evolution of the culture was monitored in real time using label-free holotomographic 3D microscopy (Supplementary videos S1-S4) [38]. Since live imaging has enabled us to observe cell death progressively, we can differentiate several apoptotic hallmarks. Membrane blebbing was observed clearly in HeLa cells treated with **11** when compared to untreated cells (Fig. 5A). The appearance of blebs at short exposure times indicates a rapid absorption of the compound by the cells since these perturbations of the membrane were not observed in the control group. Membrane blebbing is present in cells undergoing apoptosis in a characteristic way. Growth and cell movement associated blebbing appears in a polarized manner, while apoptotic cells show uniformly distributed blebs on the entire cell surface [39]. Interestingly, this effect did not occur in SW1573 cells, even when the cells eventually collapsed in the same way as HeLa cells (Fig. 5B). This result suggests a different effect on both cell lines after drug treatment [40]. After 5 h of exposure to **11** , cells started to shrink decreasing their volume and forming apoptotic bodies (Fig. 5A and B). Similar apoptotic hallmarks (e.g. induced nuclear condensation and cell shrinkage) were observed by live imaging for the known PTX, colchicine and vinblastine against SW1573 [41], and for tamoxifen against HeLa cells [42].

Live-cell imaging allows the evaluation of the phenotypic changes between subgroups of individuals of a population at single cell level and at continuous time points. This technique opens a new window in the study of cell response to potential bioactive compounds when compared to traditional studies of dose-response relationship at fixed times [41]. Thus, exposure of HeLa and SW1573 cells to **11** produces an increase of the average dry mass density (Fig. 6). This suggests the compartmentalization of cellular organelles in apoptotic bodies and the shrinkage of cells resulting in the increased dry mass density. However, this parameter decreased in the HeLa treated group after 12 h of exposure. This is related to the rupture of the membrane and the release of the cellular content to the extracellular environment, clear signal of transition into a necrotic stage. The process produces filmy bubbles in the membrane of the dead cells (Fig. 5A, upper right panel). The lack of these effects in SW1573 could be related to the time of compound exposure, indicating different potency depending on the cell subtype.

2.3. Anti-inflammatory activity of the sp^2-IGLs

The cytotoxic effect of **10** , **11** , **13** and **18** , selected as representative examples, on Bv.2 microglia cells (specific immune cells at the central nervous system) was evaluated by crystal violet staining. The reducing fluorinated sp^2-iminosugar **19** was also included in this SAR study to analyze the effect of the lipid chain. The mouse Bv.2 microglial cells were exposed for 24 h to increasing concentrations of each compound (0.1, 1, 10 and 25 μM). The cell viability remains unaltered at 10 μM in all cases, being the non-cytotoxic concentration selected to address further experiments on the anti-inflammatory activity (Fig. 7). Cytotoxic effect of different concentrations of Dexamethasone (DX) (from 0.1 to 10 μM), an anti-inflammatory reference drug used as positive control, has been included in the Supplementary Information (Fig. S1).

The stimulation of Bv.2 microglial cells by bacterial lipopolysaccharide (LPS) as external pro-inflammatory stimulus induces the

Table 2
GI_{50} (μM) values[a] of 2-deoxy-2-fluorinated-sp^2-IGLs and their 2-OH-counterparts.

Compound	Tumor cell line					
	A549 (Lung)	HBL-100 (Breast)	HeLa (Cervix)	SW1573 (Lung)	T-47D (Breast)	WiD (Colon)
Nojirimycin derivatives						
ONJ	>100	>100	>100	>100	>100	>100
19 (2-F_{eq})	>100	>100	>100	>100	>100	>100
20 (2-F_{eq}, 3-OBn)	>100	>100	>100	>100	>100	>100
23 (SC_{12})	16 ± 2	17 ± 4	17 ± 2	18 ± 1	18 ± 2	18 ± 0.2
10 (2-F_{eq}, SC_{12})	17 ± 1	22 ± 2	19 ± 1	22 ± 1	21 ± 1	21 ± 2
12 (2-F_{eq}, 3-OBn, SC_{12})	18 ± 5	19 ± 1	13 ± 5	19 ± 4	18 ± 3	20 ± 3
9 (SO_2C_{12})	22 ± 4	19 ± 2	18 ± 2	20 ± 1	21 ± 1	21 ± 1
18 (3-OBn, SO_2C_{12})	7.1 ± 1.7	15 ± 3	9.2 ± 2.7	10 ± 4	14 ± 3	13 ± 4
11 (2-F_{eq}, SO_2C_{12})	4.6 ± 0.2	19 ± 1	4.5 ± 0.3	9.1 ± 0.8	18 ± 2	20 ± 1
13 (2-F_{eq}, 3-OBn, SO_2C_{12})	4.3 ± 0.2	4.2 ± 0.4	3.3 ± 0.2	4.2 ± 0.1	9.7 ± 0.5	3.6 ± 0.1
Mannonojirimycin derivatives						
OMJ	>100	>100	>100	>100	>100	>100
21 (2-F_{ax})	>100	>100	>100	>100	>100	>100
22 (2-F_{ax}, 3-OBn)	>100	>100	>100	>100	>100	>100
24 (SC_{12})	18 ± 3	26 ± 3	19 ± 0.4	25 ± 1	25 ± 2	23 ± 2
14 (2-F_{ax}, SC_{12})	15 ± 3	18 ± 2	21 ± 3	17 ± 2	17 ± 4	16 ± 6
16 (2-F_{ax}, 3-OBn, SC_{12})	13 ± 1	18 ± 1	19 ± 6	18 ± 5	20 ± 1	18 ± 7
25 (SO_2C_{12})	18 ± 3	25 ± 3	12 ± 4	20 ± 1	23 ± 1	37 ± 15
47 (2-OMe, SO_2C_{12})	15 ± 1	20 ± 3	20 ± 6	21 ± 1	17 ± 8	21 ± 4
15 (2-F_{ax}, SO_2C_{12})	2.9 ± 0.9	7.4 ± 3.7	3.1 ± 0.5	3.6 ± 1.2	10 ± 4	4.3 ± 0.6
17 (2-F_{ax}, 3-OBn, SO_2C_{12})	4.2 ± 0.1	3.4 ± 0.4	3.6 ± 0.7	4.5 ± 0.4	3.8 ± 0.9	4.3 ± 0.7
Reference drugs[b]						
5-FU	2.2 ± 0.3	5.5 ± 2.3	15 ± 5	4.3 ± 1.6	47 ± 18	49 ± 7
CDDP	4.9 ± 0.2	1.9 ± 0.2	1.8 ± 0.5	2.7 ± 0.4	17 ± 3	23 ± 4

[a] GI_{50} values expressed in μM as means of three experiments ± standard deviation.
[b] 5-Fluorouracil (5-FU) and Cisplatin (CDDP) served as positive controls.

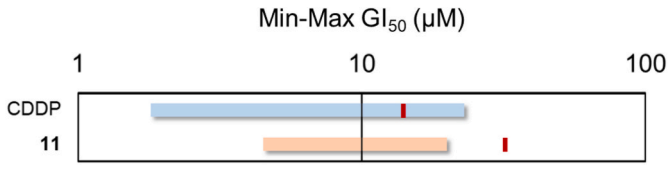


Fig. 3. GI_{50} range plot of **11** against human solid tumor cell lines (color bars) and GI_{50} against human fibroblasts (red).

synthesis and release of nitrites and pro-inflammatory cytokines. The increased nitrites expression is essential for inflammatory reactions and related processes [43]. Targeting the inducible nitric oxide synthase (iNOS), which is the enzyme responsible for nitrites synthesis, is a potential target for an anti-inflammatory therapeutic strategy [44]. The effect of these sp^2-glycomimetics on the production of nitrites

Table 3
P-gp test in SW1573 and SW1573/P-gp cell lines.[a]

Verapamil (−)				Verapamil (+)		
Compound	SW1573	SW1573/P-gp	R_f	SW1573	SW1573/P-gp	R_f[b]
11	3.3 ± 0.7	5.3 ± 0.7	1.6	15 ± 2.1	11 ± 1.5	0.7
PTX	0.00053 ± 0.00022	0.30 ± 0.11	564	0.00046 ± 0.00021	0.00031 ± 0.00015	0.7

[a] Values are given in μM and represent mean values of at least three independent experiments ± standard deviation.
[b] R_f represents the ratio between GI_{50} SW1573/P-gp and SW1573.

(pro-inflammatory mediator) after the stimulation of Bv.2 microglial cells by LPS (200 ng/mL) was examined using the Griess method. The increase of nitrites triggered by LPS was significantly reduced in all cases in the presence of 10 μM of each compound after 24 h of treatment, highlighting the fluorinated sulfonyl α-glycolipid **11** , for with which the decrease of nitrites reaches down to basal level (Fig. 8).

LPS-induced inflammatory response is characterized by releasing of pro-inflammatory cytokines such as tumor necrosis factor (TNFα) and interleukin IL-1β. These cytokines play crucial roles during inflammation and are recognized as important inflammatory mediators in M1 or pro-inflammatory microglial response [45,46]. Blockade of the production of these cytokines is responsible for the downregulation of the inflammatory process. We found that the high increase in *Tnfa and Il1b* mRNA expression in Bv.2 microglial cells after LPS stimulus was counteracted after 24 h of treatment with the sp^2-iminosugars at 10 μM (Fig. 9). Differently from that observed for the anti-proliferative activity, where the presence of the lipid chain was essential, the reducing 2-deoxy-2-fluoro-α-sp^2-iminosugar **19** was active as the lipid conjugates at decreasing the pro-inflammatory markers evaluated in this assay (nitrites, *Tnfa* and *Il1b mRNA expression*).

In order to analyze the potential M2 or anti-inflammatory enhanced response, the effect of compounds **11** and **13** on Arginase 1 (*Arg1*) mRNA expression, an anti-inflammatory marker negatively affected by the presence of LPS [47], was determined. The previously reported anti-inflammatory non-fluorinated derivative DSO_2-ONJ [26,27] was included as positive control of the induction of the M2 response. *Arg1* expression levels were greatly restored in the presence of the three sp^2-IGLs (10 μM) after treatment with LPS (Fig. 10). The body of data reveals the potential of these compounds at decreasing the pro-inflammatory response and improving the resolution of the inflammatory process.

Given the ability of DSO_2-ONJ to elicit the non-canonical activation of p38α MAPK leading to its autophosphorylation [26], the potential effect of the new fluorinated counterparts **11** and **13** on p38α MAPK phosphorylation in different time periods was assessed. Results are depicted in Fig. 11.

Treatment of Bv.2 microglial cells with LPS rapidly activated MAPKs by inducing the phosphorylation of p38α MAPK with maximal effect being elicited after 30 min. Co-treatment with **11** or **13** failed to decrease the expected phosphorylation of p38α MAPK. In the presence of LPS, the phosphorylation levels remain sustained over time with **11** (from 15 to 90 min), achieving the same effect as **13** at longer times (60–90 min). However, a differential behavior time dependent is observed for these compounds in the absence of the pro-inflammatory stimulus. The 2-fluorinated α-dodecylsulfone **11** keeps the phosphorylation profile of p38α at short times (15 and 30 min), reaching the basal level at 90 min, whereas its O3-benzylated analogue **13** maintains such profile only at 90 min at similar levels to those displayed by LPS-stimulus. In any case, both fluorinated compounds show a non-canonical anti-inflammatory effect already observed for either DSO_2-ONJ [26] or phosphatidylinositol ether lipid analogues [48].

No significant interactions between the hydroxyl group located in C2 of DSO_2-ONJ and p38α have been previously observed by computational

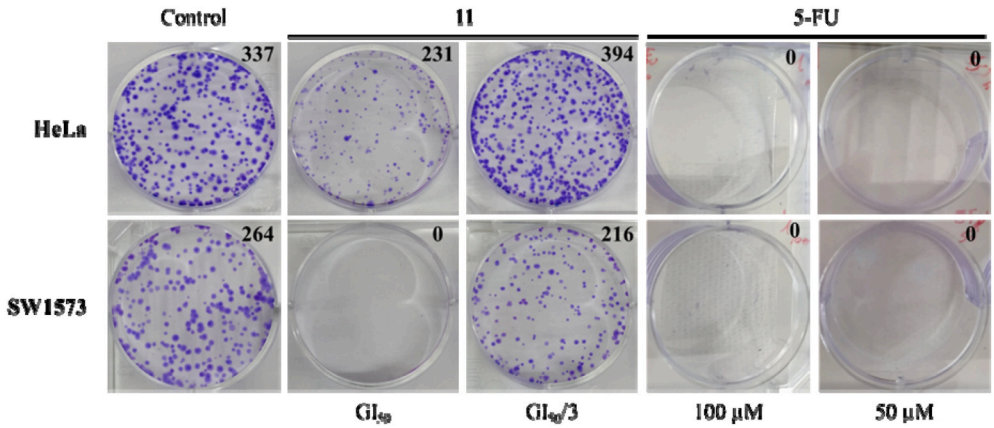


Fig. 4. Colony formation assay of HeLa and SW1573 cells in the absence (left panel) and after being exposed to compound **11** at GI_{50} or $GI_{50}/3$ concentration for 7 days. The number of colonies is shown. The anticancer drug 5-FU at 100 and 50 µM concentrations was used as positive control.

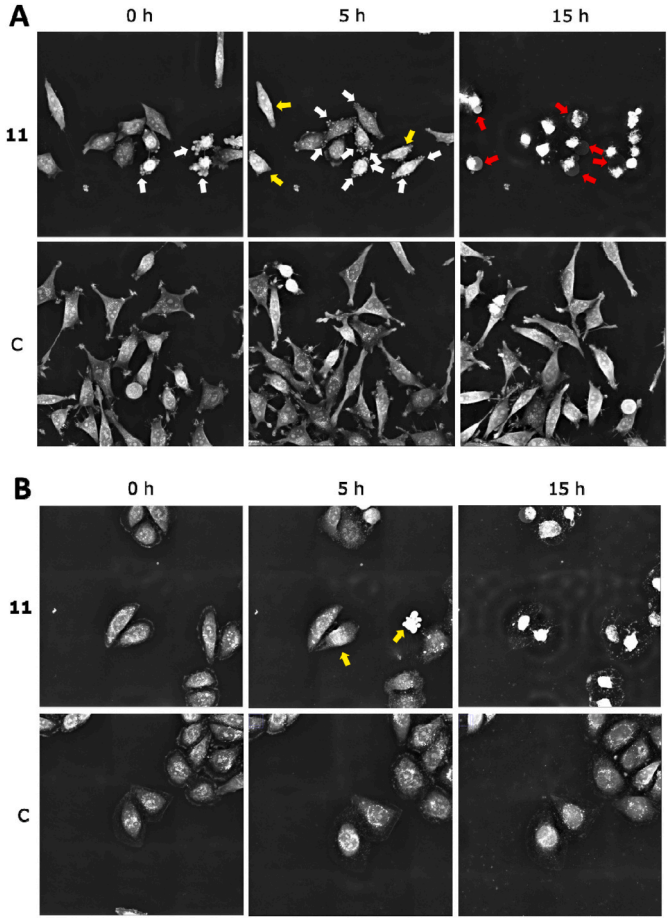


Fig. 5. Compound **11** induces apoptosis in HeLa and SW1573 cell lines. Representative images of (**A**) HeLa and (**B**) SW1573 cells incubated with or without 20 µM of **11** . White arrows: membrane blebs; Yellow arrows: cell undergoing compaction due to apoptosis; Red arrows: brighter membrane blebs, signal of possible transition to necrotic state.

studies [26]. Dissimilarly, docking experiments of **13** into the p38α structure displayed not only binding of its lipid chain into the hydrophobic pocket of the kinase, but also additional π-stacking intermolecular interactions involving the benzyl group and the amino acid tryptophan (Trp) 197, reinforcing the stability of the complex protein kinase-ligand (Fig. 12). It is interesting to speculate that the differences

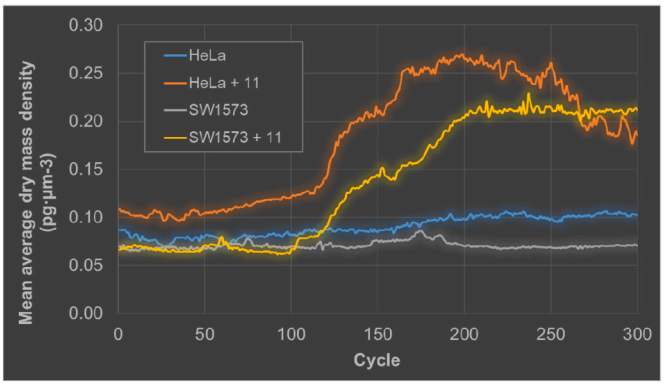


Fig. 6. Kinetics of mean average dry mass density for untreated HeLa and SW1573 cells, and cells exposed to compound **11** at 20 µM for 15 h.

in the molecular interactions at the lipid binding site of p38α might be at the origin of the disparate self-phosphorylation activation rates between the two glycolipids.

3. Conclusions

Selectfluor-mediated fluorination reaction of sp^2-iminoglycals has been employed to synthesize a novel collection of 2-deoxy-2-fluorinated-sp^2-iminosugars. The nature of the protecting group on the iminoglycal guides the F_{eq} (*gluco*)/F_{ax} (*manno*) diastereoselectivity, whereas exclusive formation of the α-anomer is warranted by the intensified anomeric effect predominant in this family of glycomimetics. The presence of the lipid chain is critical for the anti-proliferative activity of the compounds towards human cancer cell lines ($GI_{50} < 100$ µM). This activity is increased when the lipid (dodecyl) chain is linked to the glycone part through a sulfonyl group and a fluorine atom is installed at the C2 position, being associated to the induction of an apoptotic state. The anti-proliferative activity is further enhanced if an O3-benzyl substituent is present in the sp^2-IGL. Studies conducted in LPS-activated microglia additionally demonstrate an anti-inflammatory activity. Interestingly, the nature of the functional group at C2 (OH or F) and at O3 (H or Bn) has a strong influence on the ability of the sp^2-IGL to promote resolution of the inflammatory state through the non-canonic p38α MAPK phosphorylation pathway. The effect exerted by 2-deoxy-2-fluorinated-sp^2-IGLs on specific diseases with an inflammatory component such as diabetic retinopathy and nephropathy, are being currently investigated in our laboratories.

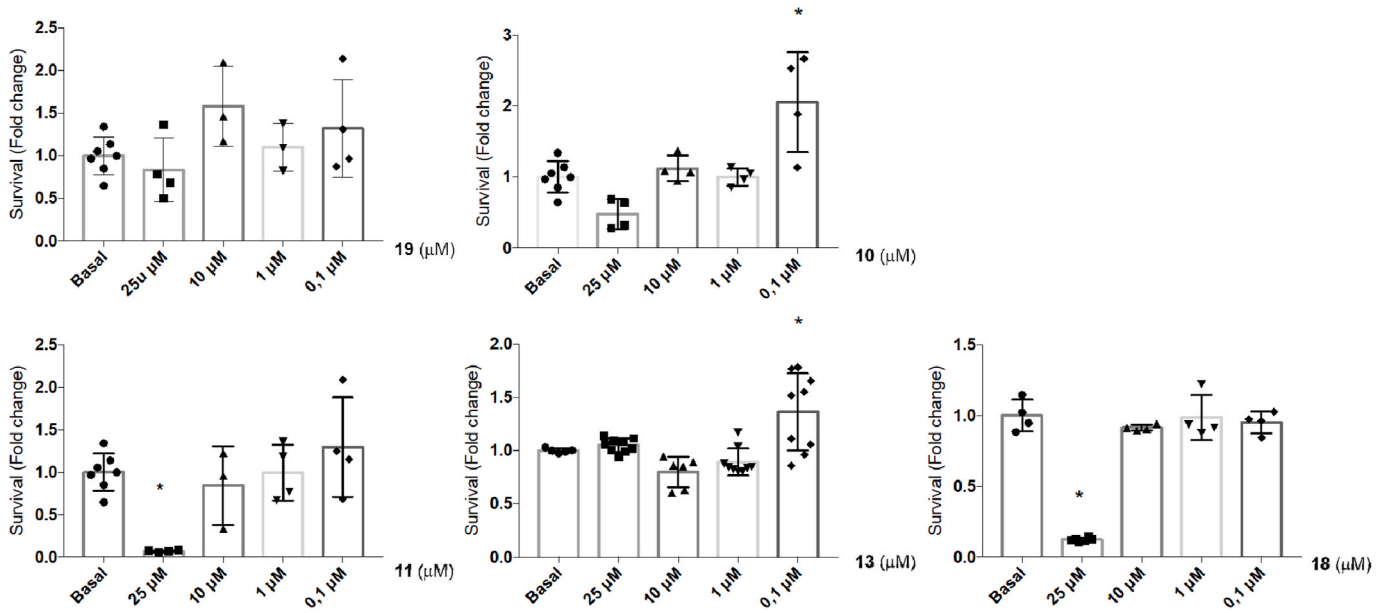


Fig. 7. Cytotoxic effect of different concentrations (from 0.1 to 25 µM) of selected sp^2-iminosugars (**10** , **11** , **13** , **18** and **19**) on the Bv.2 cell line viability. Viability was determined by crystal violet staining. Colorimetric quantification was performed, and the results are shown as mean SEM (n ≥ 3 independent experiments). The fold change relative to the basal condition is shown; *p ≤ 0.05 vs. Basal; (two-way ANOVA followed by Bonferroni *t*-test).

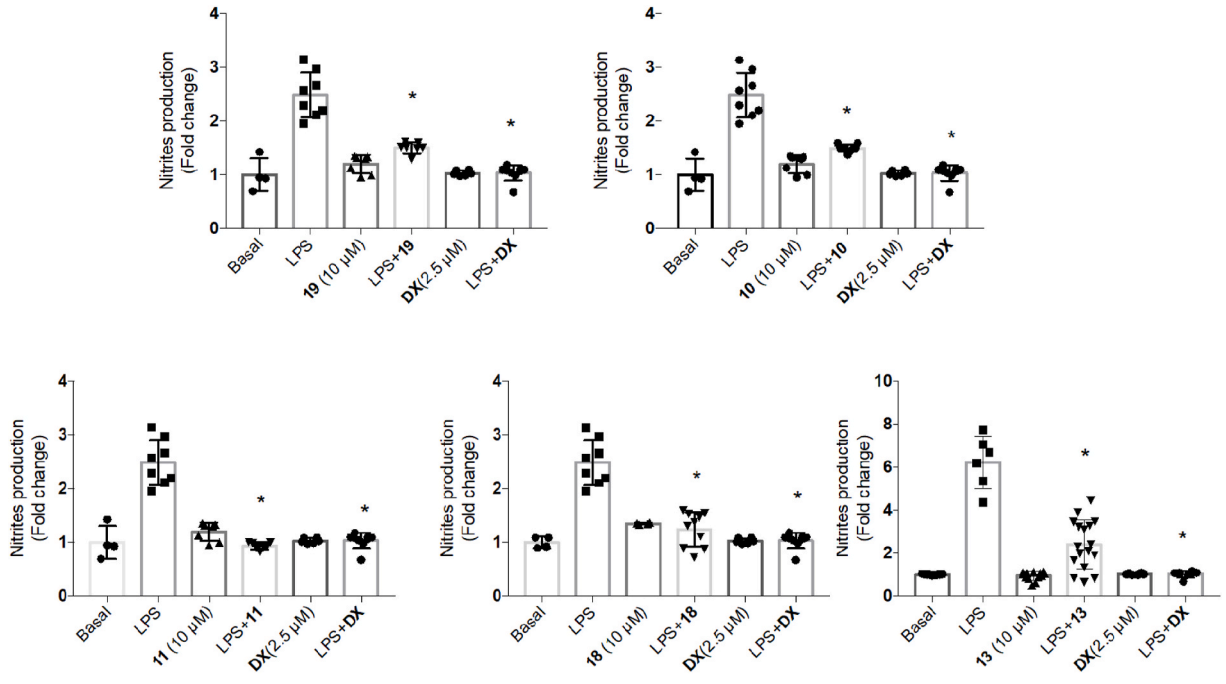


Fig. 8. Anti-inflammatory effect of selected sp^2-iminosugars (**10** , **11** , **13** , **18** and **19**) in LPS-stimulated Bv.2 cells. DX (2.5 µM) was used as positive control reducing in all cases LPS-induced nitrite production in microglia cultures. Nitrite accumulation was analyzed and related to the basal levels. Colorimetric quantification was performed. The results are expressed as mean SEM (n ≥ 4 independent experiments performed in triplicate). The fold change relative to the basal condition is shown; *p ≤ 0.05 vs. LPS (two-way ANOVA followed by Bonferroni *t*-test).

4. Experimental

4.1. General methods

All reagents and solvents were purchased from commercial sources and used without further purification. Thin-layer chromatography was performed on precoated TLC plates, silica gel 30F-245, with visualization by UV light and by carrying with 10% H_2SO_4 or 0.2% w/v cerium (IV) sulphate-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 and 35–70 µm). Optical rotations were measured at 20 ± 2 °C in 1 cm tube on a Jasco P-2000 polarimeter using a sodium lamp (λ 589 nm). NMR experiments ^1H (^{13}C and ^{19}F) were performed at 300 (75.5, 282), 400 (100.6) and 500 MHz (125.7, 470). 2D COSY and HSQC experiments were carried out to assist on NMR assignments. Abbreviations to indicate the multiplicities of the signals are: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Mass spectra were registered at the Mass Spectrometry Service of the

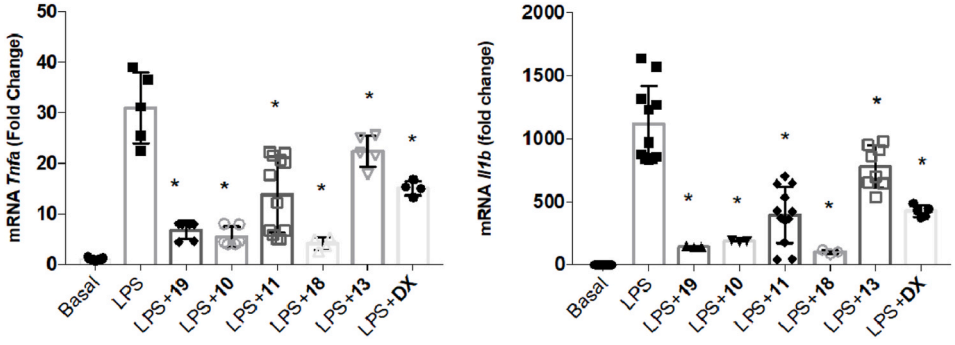


Fig. 9. Protective effect of **10** , **11** , **13** , **18** and **19** against LPS-mediated elevation of mRNA levels of pro-inflammatory cytokines (*Tnfa and Il1b*) in Bv.2 microglial cells. DX (2.5 μM) was used as positive control inhibiting mRNA expression of the classical cytokines IL1β, TNFα. mRNA levels were determined by qRT-PCR after treatment and *Actinb* was used as housekeeping control (n ≥ 4 independent experiments performed in triplicate). The fold change relative to the basal condition is shown; *p ≤ 0.05 vs. LPS condition (two-way ANOVA followed by Bonferroni *t*-test).

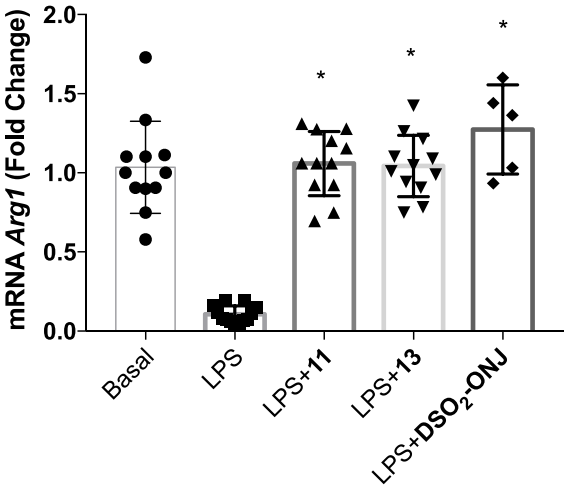


Fig. 10. The anti-inflammatory response mediated by Arginase-1 expression. Evaluation of the effect of **11** , **13** on the *Arg1* expression with LPS. The representative sp^2-iminosugar-based glycomimetic DSO_2-ONJ was included as positive control. Bv.2 microglial cells were stimulated with 200 ng/ml LPS in the absence or presence of **11** , **13** and DSO_2-ONJ (10 μM) for 24 h mRNA levels were determined by qRT-PCR after treatment and *Actinb* was used as housekeeping control (n ≥ 4 independent experiments performed in triplicate). The fold change relative to the basal condition is shown; *p ≤ 0.05 vs. LPS condition (two-way ANOVA followed by Bonferroni *t*-test).

Institute for Chemical Research (unit 824861, IIQ, CSIC-US, Spain) using a *Bruker Elute UHPLC* system coupled to a *Bruker AmaZon SL* spectrometer instrument. The system is equipped with an ESI (Electrospray Ionization) ionization source and a Dual Fuel Ion Trap. Samples (2–5 μL of 10–20 μM solutions) were either directly injected or chromatographed using 0.1% formic acid eluting gradients at a flow rate of 0.3 mL/min. Spectra were registered in both positive and negative modes and the results were processed using *Bruker Compass HyStar* software in the *m/z* 100–2000 range. All compounds were purified to ≥95% purity as determined by elemental microanalysis results obtained on an elemental analyser Leco CHNS-932 (IIQ, CSIC-US). The analytical results for C, H, N, and S were within ±0.4 of the theoretical values. Deprotection reactions of benzoyl or acetyl protecting groups were carried out by using Zemplén conditions [33]. Addition of NaOMe (1 M) (0.1 equiv/Bz mol or Ac mol) in MeOH at room temperature, followed by neutralization with solid CO_2, evaporation of the solvent and purification by column chromatography. 3,4-Di-*O*-acetyl-5*N*,6*O*-(oxomethylidene)nojirimycin iminoglycal (**26**) [32], 5*N*,6*O*-oxomethylidenenojirimycin iminoglycal (**29**) [34], 3,4-di-*O*-benzyl-5*N*,6*O*-oxomethylidenenojirimycin iminoglycal (**32**) [34], 5-azido-3-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (**36**) [35] were prepared according to previously reported procedures.

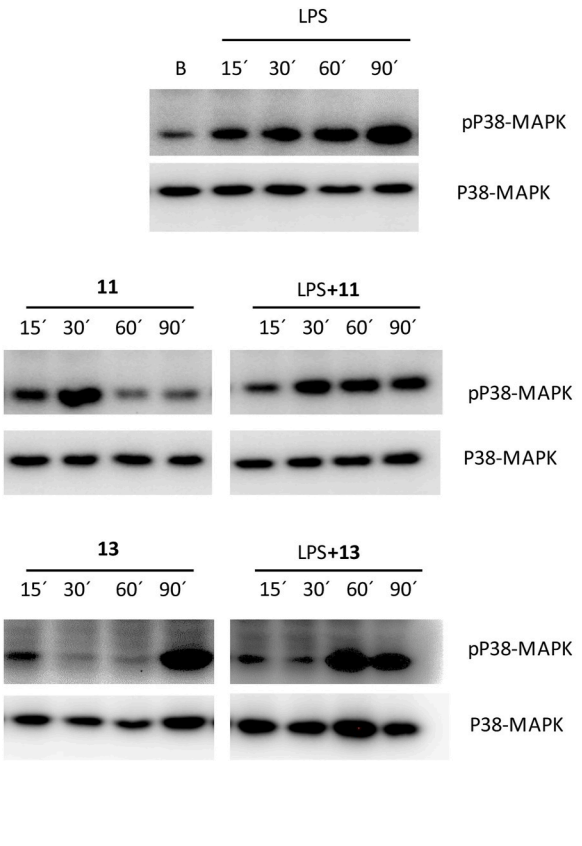
4.2. Synthesis

4.2.1. Synthesis of new protected sp^2-iminoglycals (**30** , **31** , **33** , **34**)

3,4-Di-*O*-benzoyl-5*N*,6*O*-oxomethylidenenojirimycin iminoglycal (30). Benzoyl chloride (395 μL, 3.39 mmol) was added over a stirred solution of 5*N*,6*O*-oxomethylidenenojirimycin iminoglycal (**29**) (194 mg, 1.13 mmol) in pyridine (5.7 mL) at 0 °C under Ar atmosphere. The reaction mixture was stirred at room temperature for 18 h, diluted with ice-water (15 mL) and CH_2Cl_2 (50 mL), washed with 1N HCl (3 × 25 mL), aqueous $NaHCO_3$ (3 × 25 mL) and water (2 × 10 mL), dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified by column chromatography (1:4 → 1:2 EtOAc-cyclohexane). Yield: 351 mg (82%). R_f 0.38 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ −147.4 (*c* 1.2 in CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ 8.05–7.30 (m, 10 H, Ph), 6.79 (dd, 1 H, $J_{1,2}$ = 7.9 Hz, $J_{1,3}$ = 1.7 Hz, H-1), 6.10 (dt, 1 H, $J_{3,4}$ = 8.0 Hz, $J_{2,3}$ = 2.0 Hz, H-3), 5.63 (dd, 1 H, $J_{4,5}$ = 10.3 Hz, H-4), 5.14 (dd, 1 H, H-2), 4.56 (t, 1 H, $J_{6a,6b}$ = $J_{5,6a}$ = 8.4 Hz, H-6a), 4.46 (t, 1 H, $J_{5,6b}$ = 9.0 Hz, H-6b), 4.44–4.30 (m, 1 H, H-5). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 166.1–165.8 (CO ester), 153.1 (CO), 133.9–128.4 (Ph), 123.8 (C-1), 105.8 (C-2), 71.6 (C-4), 70.7 (C-3), 67.2 (C-6), 54.5 (C-5). ESIMS: *m/z* 402.17 $[M + Na]^+$. Anal. Calcd for $C_{21}H_{17}NO_6$: C, 66.49; H, 4.52; N, 3.69. Found: C, 66.57; H, 4.61; N, 3.62.

3,4-Di-*O*-pivaloyl-5*N*,6*O*-oxomethylidenenojirimycin iminoglycal (31). To a stirred solution of 5*N*,6*O*-oxomethylidenenojirimycin iminoglycal (**29**) (53 mg, 0.31 mmol) in THF (1.7 mL) under Ar atmosphere, 4-dimethylaminopyridine (152 mg, 1.24 mmol) and pivaloyl chloride (153 μL, 1.24 mmol) were added at 0 °C. The reaction mixture was stirred at room temperature for 18 h, diluted with CH_2Cl_2 (25 mL) and washed with water (2 × 15 mL), dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified by column chromatography (1:3 EtOAc-cyclohexane → EtOAc). Yield: 90 mg (86%). R_f 0.57 (1:3 EtOAc-cyclohexane). $[\alpha]_D$ −56.9 (*c* 1.1 in CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ 6.69 (dd, 1 H, $J_{1,2}$ = 8.0 Hz, $J_{1,3}$ = 2.0 Hz, H-1), 5.67 (dt, 1 H, $J_{3,4}$ = 8.0 Hz, $J_{2,3}$ = 2.0 Hz, H-3), 5.24 (dd, 1 H, $J_{4,5}$ = 10.4 Hz, H-4), 4.91 (dd, 1 H, H-2), 4.45 (t, 1 H, $J_{6a,6b}$ = $J_{5,6a}$ = 8.5 Hz, H-6a), 4.26 (t, 1 H, $J_{5,6b}$ = 9.5 Hz, H-6b), 4.16 (td, 1 H, H-5), 1.18–1.17 (2 s, 18 H, Me_3CCO). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 177.9–177.6 (Me_3CCO), 153.0 (CO), 123.4 (C-1), 106.1 (C-2), 70.6 (C-4), 69.7 (C-3), 66.9 (C-6), 54.2 (C-5), 38.9–38.8 (Me_3CCO), 27.0 (Me_3CCO). ESIMS: *m/z* 338.4 $[M - H]^-$. Anal. Calcd for $C_{17}H_{25}NO_6$: C, 60.16; H, 7.43; N, 4.13. Found: C 60.20, H 7.35, N 4.00.

3,4-*O*-(*o*-Xylylenyl)-5*N*,6*O*-oxomethylidenenojirimycin iminoglycal (33). To a solution of 5*N*,6*O*-oxomethylidenenojirimycin iminoglycal (**29**) (55 mg, 0.32 mmol) in dry DMF (6.4 mL) under Ar atmosphere, NaH (90%, 31 mg, 1.28 mmol) was added at 0 °C, and the suspension was stirred for 15 min. Then, a solution of 1,2-bis(bromomethyl)bencene (170 mg, 0.64 mmol) in dry DMF (3 mL) was added dropwise, and the reaction mixture was stirred for 20 h at room temperature. After quenching with MeOH (14 mL), the solvent was removed under reduced pressure. Then, water (15 mL) and a mixture of toluene/diethyl ether was added (1:1, 20 mL), the organic layer was washed with



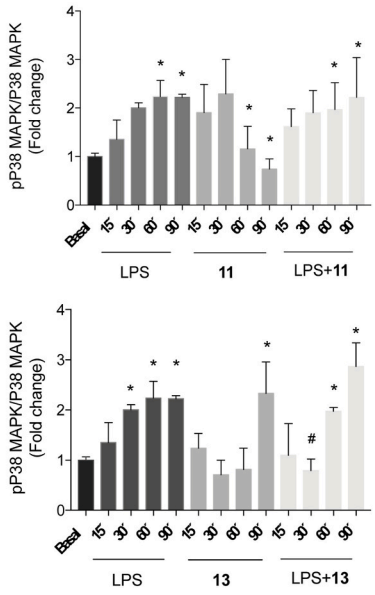


Fig. 11. Effects of **11** and **13** in the activation of MAPKs-mediated signaling in LPS-stimulated Bv.2 microglial cells. Bv.2 microglial cells were stimulated with 200 ng/mL LPS in the absence, or presence, of **11** and **13** (10 μM) for the indicated time periods. Protein extracts were separated by SDS-PAGE and analyzed by Western blot with antibodies against phosphorylated (p)-p38α MAPK and total p38α MAPK. Representative autoradiograms are shown (n = 3 independent experiments). Blots were quantified with scanning densitometry, and the results are presented as mean ± SEM. The ratios between the indicated proteins and the fold changes relative to the basal values are shown. *p ≤ 0.05 vs Basal treatment; # vs LPS treatment (two-way ANOVA followed by Bonferroni *t*-test).

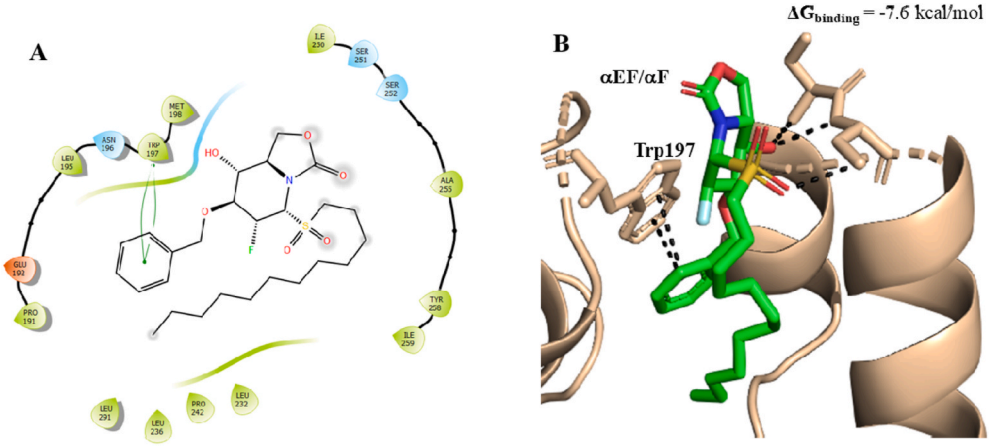


Fig. 12. Docking experiments of **13** to p38α MAPK using the protein coordinates from the PDB ID 4E6A. Molecular representations of the hydrophobic pocket: **A**) Software Maestro; **B**) Software PyMol.

brine (2 × 15 mL), dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified by column chromatography (1:2 EtOAc-cyclohexane). Yield: 58 mg (66%). R_f 0.75 (1:3 EtOAc-cyclohexane). $[\alpha]_D$ +12.3 (*c* 1.0 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.05 (m, 4 H, Ar), 6.48 (dd, 1 H, $J_{1,2}$ = 7.8 Hz, $J_{1,3}$ = 2.0 Hz, H-1), 5.03 (d, 1 H, $^2J_{H,H}$ = 12.9 Hz, OC*H*Ar), 4.99 (d, 1 H, $^2J_{H,H}$ = 14.3 Hz, OC*H*Ar), 4.88 (d, 1 H, $^2J_{H,H}$ = 14.3 Hz, OC*H*Ar), 4.85 (d, 1 H, $^2J_{H,H}$ = 12.9 Hz, OC*H*Ar), 4.89–4.85 (m, 1 H, H-2), 4.54 (t, 1 H, $J_{6a,6b}$ = $J_{5,6a}$ = 8.5 Hz, H-6a), 4.27 (dt, 1 H, $J_{3,4}$ = 7.4 Hz, $J_{2,3}$ = 2.0 Hz, H-3), 4.10 (t, 1 H, $J_{5,6b}$ = 9.0 Hz, H-6b), 3.87 (bq, 1 H, $J_{4,5}$ = 9.3 Hz, H-5), 3.63 (dd, 1 H, H-4). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 153.4 (CO), 137.0–128.2 (Ar), 122.3 (C-1), 108.8 (C-2), 78.2 (C-4), 77.0 (C-3), 71.7 (OCH_2), 67.5 (C-6), 54.3 (C-5). ESIMS: *m/z* 296.13 $[M + Na]^+$. Anal. Calcd for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53; N, 5.13. Found: C 66.19, H 5.72, N 4.98.

*4.2.1.1. Synthesis of the precursors of 4-O-acetyl-3-O-benzyl-5N,6O-oxomethylidenenojirimycin iminoglycal (**34**) from 5-azido-3-O-benzyl-5-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (**36**).* **5-Amino-3-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (37)** . To a stirred solution of the 5-azido-derivative **36** (190 mg, 0.57 mmol) in dioxane/methanol (5:1, 9 mL), Ph_3P (526 mg, 1.98 mmol) was added, and the reaction mixture was stirred at room temperature for 5 h. Next, NH_4OH (0.8 mL) was added, and the mixture was stirred for 24 h, the solvent was removed under reduced pressure and the resulting residue was purified by column chromatography (EtOAc → 45:5:1 → 45:5:3 EtOAc-EtOH-H_2O). Yield: 158 mg (90%). R_f 0.41 (45:5:3 EtOAc-EtOH-

H_2O). $[\alpha]_D$ −68.2 (*c* 0.8 in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.45–7.30 (m, 5 H, Ph), 5.94 (d, 1 H, $J_{1,2} = 3.8$ Hz, H-1), 4.76 (d, 1 H, $^2J_{H,H} = 11.8$ Hz, OC*H*$_2$Ph), 4.64 (d, 1 H, H-2), 4.51 (d, 1 H, OC*H*$_2$Ph), 4.03 (d, 1 H, $J_{3,4} = 3.0$ Hz, H-3), 3.99 (dd, 1 H, $J_{4,5} = 8.4$ Hz, H-4), 3.79 (dd, 1 H, $J_{6a,6b} = 10.8$ Hz, $J_{5,6a} = 4.0$ Hz, H-6a), 3.58 (dd, 1 H, $J_{5,6b} = 6.0$ Hz, H-6b), 3.29–3.21 (m, 1 H, H-5), 2.00 (bs, 1 H, OH), 1.50, 1.34 (2 s, 6 H, CMe_2). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 137.1–128.0 (Ph), 111.7 (*C*Me_2), 105.2 (C-1), 81.9 (C-2), 81.6–81.5 (C-3, C-4), 71.7 (O*C*H$_2$Ph), 64.9 (C-6), 50.9 (C-5), 26.7–26.2 (C*Me*$_2$). ESIMS: *m/z* 310.1 $[M + H]^+$. Anal. Calcd for $C_{16}H_{23}NO_5$: C 62.12, H 7.49, N 4.53. Found: C 61.88, H 7.27, N 4.44.

5-Amino-3-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α- D -gluco-furanose 5,6-(cyclic carbamate) (38) . To a stirred solution of the amine **37** (134 mg, 0.43 mmol) in CH_2Cl_2 (7 mL), DIPEA (0.7 mL, 4.30 mmol) and triphosgene (195 mg, 0.65 mmol) were added at 0 °C. After 15 min the solvent was removed under reduced pressure, and the residue was purified by column chromatography (EtOAc). Yield: 95 mg (66%). R_f 0.84 (EtOAc). $[\alpha]_D$ −91.7 (*c* 1.0 in $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.50–7.30 (m, 5 H, Ph), 5.95 (d, 1 H, $J_{1,2} = 3.8$ Hz, H-1), 5.38 (bs, 1 H, NH), 4.74 (d, 1 H, $^2J_{H,H} = 11.8$ Hz, OC*H*$_2$Ph), 4.68 (d, 1 H, H-2), 4.51 (d, 1 H, OC*H*$_2$Ph), 4.51–4.46 (m, 1 H, H-6a), 4.43 (dd, 1 H, $J_{6a,6b} = 8.8$ Hz, $J_{5,6b} = 4.8$ Hz, H-6b), 4.20 (dd, 1 H, $J_{4,5} = 7.8$ Hz, $J_{3,4} = 3.4$ Hz, H-4), 4.12–4.05 (m, 1 H, H-5), 4.02 (d, 1 H, H-3), 1.52–1.35 (2 s, 6 H, CMe_2). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 160.0 (CO), 137.1–128.0 (Ph), 112.2 (*C*Me_2), 105.5 (C-1), 82.0 (C-2), 81.6 (C-4), 81.3 (C-3), 71.8 (O*C*H$_2$Ph), 68.1 (C-6), 50.8 (C-5), 26.9, 26.3 (C*Me*$_2$). ESIMS: *m/z* 358.1 $[M + Na]^+$. Anal. Calcd for $C_{17}H_{21}NO_6$: C 60.89, H 6.31, N 4.18. Found: C 60.93, H 6.24, N 4.09.

(1*R*)-3-*O*-Benzyl-5*N*,6*O*-oxomethylidenenojirimycin (39). A solution of the 5,6-cyclic carbamate **38** (500 mg, 1.49 mmol) in 90% TFA-H_2O (5.0 mL) was stirred at room temperature for 2 h. The solvent was eliminated under reduced pressure, coevaporated with water, neutralized with 0.1 N NaOH and concentrated under reduced pressure. The resulting residue was purified by column chromatography using EtOAc as eluent, concentrated and freeze-dried. Yield: 400 mg (91%). R_f 0.60 (EtOAc). $[\alpha]_D$ −2.7 (*c* 1.0 in H_2O). ^1H NMR (400 MHz, D_2O) δ 7.60–7.41 (m, 5 H, Ph), 5.42 (d, 1 H, $J_{1,2} = 4.0$ Hz, H-1), 4.93 (d, 1 H, $^2J_{H,H} = 10.8$ Hz, OC*H*$_2$Ph), 4.89 (d, 1 H, OC*H*$_2$Ph), 4.66 (t, 1 H, $J_{6a,6b} = J_{5,6a} = 8.8$ Hz, H-6a), 4.34 (dd, 1 H, $J_{5,6b} = 6.8$ Hz, H-6b), 4.07–3.98 (m, 1 H, H-5), 3.78 (t, 1 H, $J_{2,3} = J_{3,4} = 9.4$ Hz, H-3), 3.71 (dd, 1 H, H-2), 3.65 (t, 1 H, $J_{4,5} = 9.4$ Hz, H-4). ^{13}C NMR (100.6 MHz, D_2O) δ 157.6 (CO), 137.6–128.4 (Ph), 80.9 (C-3), 75.3 (O*C*H$_2$Ph), 74.6 (C-1), 73.3 (C-4), 71.1 (C-2), 67.6 (C-6), 53.2 (C-5). ESIMS: *m/z* 318.1 $[M + Na]^+$. Anal. Calcd for $C_{14}H_{17}NO_6$: C 56.94, H 5.80, N 4.74. Found: C 56.75, H 5.63, N 4.57.

(1*R*)-1,2,4-Tri-*O*-acetyl-3-*O*-benzyl-5*N*,6*O*-oxomethylidenenojirimycin (40). Over a solution of **39** (2.55 g, 8.64 mmol) in pyridine (9 mL), Ac_2O (9 mL) was added at 0 °C and the mixture was stirred at room temperature for 24 h, diluted with ice-water (25 mL) and CH_2Cl_2 (80 mL), washed with 1 N HCl (3 × 25 mL), aqueous $NaHCO_3$ (3 × 25 mL) and water (2 × 10 mL), dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified by column chromatography (1:2 → 1:1 EtOAc-cyclohexane). Yield: 2.62 g (72%). R_f 0.37 (EtOAc-cyclohexane 1:1). $[\alpha]_D$ +13.5 (*c* 1.0 in CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ 7.38–7.11 (m, 5 H, Ph), 6.63 (d, 1 H, $J_{1,2} = 3.9$ Hz, H-1), 5.00 (dd, 1 H, $J_{2,3} = 10.0$ Hz, H-2), 4.87 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 4.66 (d, 1 H, $^2J_{H,H} = 11.7$ Hz, OC*H*$_2$Ph), 4.60 (d, 1 H, OC*H*$_2$Ph), 4.32 (t, 1 H, $J_{6a,6b} = J_{5,6a} = 9.0$ Hz, H-6a), 4.22 (t, 1 H, $J_{5,6b} = 9.0$ Hz, H-6b), 3.94–3.83 (m, 2 H, H-3, H-5), 2.05–1.91 (3 s, 9 H, MeCO). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.2–168.6 (Me*CO*), 154.5 (CO), 137.8–127.5 (Ph), 76.8 (C-3), 75.6 (OCH_2Ph), 73.9 (C-4), 72.9 (C-1), 71.2 (C-2), 67.2 (C-6), 53.1 (C-5), 20.6 (*Me*CO). ESIMS: *m/z* 466.30 $[M + HCO_2]^-$. Anal. Calcd for $C_{20}H_{33}NO_9$: C 57.00, H 5.50, N 3.32. Found: C 57.09, H 5.64, N 3.18.

4-*O*-Acetyl-3-*O*-benzyl-5*N*,6*O*-oxomethylidenenojirimycin iminoglycal (34). To a solution of the protected sp^2-iminosugar **40** (760 mg, 1.80 mmol) in dry CH_2Cl_2 (3 mL), HBr/AcOH (33%) (1.2 mL) was added dropwise at 0 °C and the reaction mixture was stirred for 10 min, diluted with CH_2Cl_2 (50 mL), washed with aqueous $NaHCO_3$ (2 × 20 mL), dried ($MgSO_4$), filtered and concentrated. The bromo-derivative was used in the next step without further purification. Cp_2TiCl_2 (449 mg, 1.80 mmol) and Mn (257 mg, 4.68 mmol) were dissolved in dry and deoxygenated THF (10 mL) under Ar atmosphere and stirred at room temperature until the red solution turned green. Next, the glycosyl bromide dissolved in dry and deoxygenated THF (20 mL) was added dropwise to the green solution and the reaction mixture was stirred for 1 h. The solvent was removed under reduced pressure and the resulting residue was redissolved in EtOAc (50 mL), washed with a solution of 0.1 N HCl (3 × 15 mL), dried ($MgSO_4$), filtered and concentrated. The crude was purified by column chromatography (1:3 ⟶ 1:1 EtOAc-cyclohexane). Yield: 425 mg (78%). R_f 0.56 (1:1 EtOAc-cyclohexane). $[\alpha]_D$ −60.1 (*c* 1.0 in CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.10 (m, 5 H, Ph), 6.55 (dd, 1 H, $J_{1,2} = 8.0$ Hz, $J_{1,3} = 1.8$ Hz, H-1), 5.09 (dd, 1 H, $J_{4,5} = 10.6$ Hz, $J_{3,4} = 7.9$ Hz, H-4), 5.02 (dd, 1 H, $J_{2,3} = 2.0$ Hz, H-2), 4.58 (d, 1 H, $^2J_{H,H} = 11.9$ Hz, OC*H*$_2$Ph), 4.45 (d, 1 H, OC*H*$_2$Ph), 4.36 (t, 1 H, $J_{6a,6b} = J_{5,6a} = 8.5$ Hz, H-6a), 4.28 (dt, 1 H, H-3), 4.18 (t, 1 H, $J_{5,6b} = 9.0$ Hz, H-6b), 3.96 (td, 1 H, H-5), 1.99 (s, 3 H, MeCO). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.2 (Me*CO*), 153.2 (CO), 137.7–127.8 (Ph), 122.6 (C-1), 107.1 (C-2), 75.1 (C-3), 71.9 (C-4), 70.9 (OCH_2Ph), 67.2 (C-6), 54.3 (C-5), 20.8 (*Me*CO). ESIMS: *m/z* 326.1 $[M + Na]^+$. Anal. Calcd for $C_{16}H_{17}NO_5$: C, 63.36; H, 5.65; N, 4.62. Found: C, 63.47; H, 5.79; N, 4.50.

4.2.2. General procedure for the fluorination reaction of the sp^2-iminoglycals mediated by Selectfluor^R

To a stirred solution of the corresponding sp^2-iminoglycal derivative (**26** , **30** , **34**) (0.66 mmol) in nitromethane-water (1.5 mL–0.3 mL), Selectfluor^R (300 mg) was added at 0 °C and the reaction mixture was stirred at room temperature for 20 h. Then, the solvents were removed under reduced pressure and the residue was diluted with CH_2Cl_2 (50 mL) and washed with 5% $NaHCO_3$ solution (15 mL), brine (15 mL), dried ($MgSO_4$), filtered and concentrated. Subsequently, the resulting crude was dissolved in dry CH_2Cl_2 (1.7 mL), and dry pyridine (102 μL) and Ac_2O (75 μL) were added at room temperature stirring the reaction mixture for 20 h, washed with saturated $NaHCO_3$ (15 mL) and water (2 × 10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a mixture of fluorinated C2 epimeric diasteromers. Purification by column chromatography using the solvents indicated in each case afforded the corresponding 2-deoxy-2-fluoro derivatives with *gluco* (**27** , **41** , **52**) and *manno* (**28** , **42** , **53**) configurations.

(1*R*)-1,3,4-Tri-*O*-acetyl-2-deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-nojirimycin (27). Purification by column chromatography (2:3 → 1:1 → 2:1 EtOAc-cyclohexane). Yield: 70 mg (32%). R_f 0.44 (2:1 EtOAc-cyclohexane). $[\alpha]_D$ +29.3 (*c* 0.8 in CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ 6.80 (d, 1 H, $J_{1,2} = 3.6$ Hz, H-1), 5.53 (q, 1 H, $J_{2,3} = J_{3,4} = J_{3,F} = 9.6$ Hz, H-3), 4.94 (t, 1 H, $J_{4,5} = 9.6$ Hz, H-4), 4.65 (ddd, 1 H, $J_{2,F} = 47.7$ Hz, H-2), 4.42 (dd, 1 H, $J_{6a,6b} = 9.0$ Hz, $J_{5,6a} = 8.4$ Hz, H-6a), 4.23 (dd, 1 H, $J_{5,6b} = 7.2$ Hz, H-6b), 4.00 (bq, 1 H, H-5), 2.13–2.04 (3 s, 9 H, MeCO). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.0–168.3 (Me*CO*), 154.2 (CO), 86.2 (C-2, d, $J_{C2,F} = 196.8$ Hz), 71.7 (C-1, d, $J_{C1,F} = 16.5$ Hz), 71.5 (C-4), 69.7 (C-3, d, $J_{C3,F} = 20.2$ Hz), 66.6 (C-6), 52.2 (C-5), 20.6 (*Me*CO). ^{19}F NMR (282 MHz, $CDCl_3$) δ −200.4. ESIMS: *m/z* 356.1 $[M + Na]^+$. Anal. Calcd for $C_{13}H_{16}FNO_8$: C, 46.85; H, 4.84; F, 5.70; N, 4.20. Found: C, 46.91; H, 4.77; N, 4.08.

(1*R*)-1,3,4-Tri-*O*-acetyl-2-deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-mannonojirimycin (28). Purification by column chromatography (2:3 → 1:1 → 2:1 EtOAc-cyclohexane). Yield: 105 mg (48%). R_f 0.31 (2:1 EtOAc-cyclohexane). $[\alpha]_D$ +11.5 (*c* 1.0 in CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ 6.55 (dd, 1 H, $J_{1,F} = 6.3$ Hz, $J_{1,2} = 3.0$ Hz, H-1), 5.40–5.15 (m, 2 H, H-3, H-4), 4.88 (bd, 1 H, $J_{2,F} = 47.7$ Hz, H-2), 4.45 (t, 1 H, $J_{6a,6b} = J_{5,6a} = 9.5$ Hz, H-6a), 4.34 (dd, 1 H, $J_{5,6b} = 7.0$ Hz, H-6b), 4.01 (bq, 1 H, $J_{4,5} = 8.4$ Hz, H-5), 2.14–2.08 (3 s, 9 H, MeCO). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 169.9–168.1 (Me*CO*), 154.9 (CO), 85.6 (C-2, d, $J_{C2,F} = 184.9$ Hz), 74.2 (C-1, d, $J_{C1,F} = 30.8$ Hz), 69.1–68.9 (C-3, C-4), 66.6 (C-6), 53.3 (C-5), 20.6–20.5 (*Me*CO). ^{19}F NMR (282 MHz, $CDCl_3$) δ

−201.5. ESIMS: *m/z* 356.1 $[M + Na]^+$. Anal. Calcd for $C_{13}H_{16}FNO_8$: C, 46.85; H, 4.84; F, 5.70; N, 4.20. Found: C, 47.00; H, 4.88; N, 4.12.

(1*R*)-1-*O*-Acetyl-3,4-di-*O*-benzoyl-2-deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-nojirimycin (41). Purification by column chromatography (2:3 → 1:1 → 2:1 EtOAc-cyclohexane). Yield: 136 mg (45%). R_f 0.35 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ −0.9 (*c* 1.0 in CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ 7.97–7.35 (m, 10 H, Ph), 6.98 (dd, 1 H, $J_{1,2} = 4.2$ Hz, $J_{1,F} = 1.0$ Hz, H-1), 5.99 (q, 1 H, $J_{3,F} = J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.34 (t, 1 H, $J_{4,5} = 9.7$ Hz, H-4), 4.90 (ddd, 1 H, $J_{2,F} = 47.8$ Hz, H-2), 4.57–4.44 (m, 2 H, H-6a, H-6b), 4.21 (td, 1 H, $J_{5,6a} = 9.4$ Hz, $J_{5,6b} = 7.4$ Hz, H-5), 2.23 (s, 3 H, MeCO). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.4–165.7 (Ph*CO,* Me*CO*), 154.4 (CO), 134.2–128.1 (Ph), 86.6 (C-2, d, $J_{C2,F} = 197.3$ Hz), 77.4 (C-4, d, $J_{C4,F} = 7.1$ Hz), 72.0 (C-1, d, $J_{C1,F} = 24.5$ Hz), 70.2 (C-3, d, $J_{C3,F} = 20.1$ Hz), 67.0 (C-6), 53.0 (C-5), 20.8 (*Me*CO). ^{19}F NMR (282 MHz, $CDCl_3$) δ −200.0. ESIMS: *m/z* 480.29 $[M + Na]^+$. Anal. Calcd for $C_{23}H_{20}FNO_8$: C, 60.39; H, 4.41; N, 3.06. Found: C, 60.13; H, 4.19; N, 2.83.

(1*R*)-1-*O*-Acetyl-3,4-di-*O*-benzoyl-2-deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-mannonojirimycin (42). Purification by column chromatography (2:3 → 1:1 → 2:1 EtOAc-cyclohexane). Yield: 81 mg (27%). R_f 0.28 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ −41.1 (*c* 1.2 in CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ 8.00–7.32 (m, 5 H, Ph), 6.67 (dd, 1 H, $J_{1,F} = 6.2$ Hz, $J_{1,2} = 2.9$ Hz, H-1), 5.72 (ddd, 1 H, $J_{3,F} = 31.5$ Hz, $J_{3,4} = 10.2$ Hz, $J_{2,3} = 2.5$ Hz, H-3), 5.71–5.61 (m, 1 H, H-4), 5.08 (dt, 1 H, $J_{2,F} = 48.3$ Hz, H-2), 4.52 (d, 2 H, $J_{5,6} = 7.5$ Hz, H-6a), 4.23 (bq, 1 H, $J_{4,5} = 8.0$ Hz, H-5), 2.20 (s, 3 H, MeCO). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.2–165.6 (Ph*CO*, Me*CO*), 155.0 (CO), 85.9 (C-2, d, $J_{C2,F} = 185.7$ Hz), 74.4 (C-1, d, $J_{C1,F} = 31.0$ Hz), 69.7–69.5 (C-3, C-4), 66.9 (C-6), 53.7 (C-5), 20.7 (*Me*CO). ^{19}F NMR (282 MHz, $CDCl_3$) δ −201.2. ESIMS: *m/z* 937.31 $[2M + Na]^+$. Anal. Calcd for $C_{23}H_{20}FNO_8$: C, 60.39; H, 4.41; N, 3.06. Found: C, 60.15; H, 4.22; N, 2.84.

(1*R*)-1,4-Di-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-nojirimycin (52). Purification by column chromatography (1:3 → 1:1 EtOAc-cyclohexane). Yield: 85 mg (34%). R_f 0.35 (1:1 EtOAc-cyclohexane). $[\alpha]_D$ +25.0 (*c* 1.0 in CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.20 (m, 5 H, Ph), 6.82 (dd, 1 H, $J_{1,2} = 4.3$ Hz, $J_{1,F} = 1.7$ Hz, H-1), 4.89 (t, 1 H, $J_{4,5} = J_{3,4} = 10.0$ Hz, H-4), 4.88 (d, 1 H, $^2J_{H,H} = 11.8$ Hz, OCH_2Ph), 4.69 (ddd, 1 H, $J_{2,F} = 47.7$ Hz, $J_{2,3} = 9.2$ Hz, H-2), 4.64 (d, 1 H, OCH_2Ph), 4.39 (dd, 1 H, $J_{6a,6b} = 9.2$ Hz, $J_{5,6a} = 8.2$ Hz, H-6a), 4.26 (dd, 1 H, $J_{5,6b} = 7.4$ Hz, H-6b), 4.05–3.85 (m, 2 H, H-3, H-5), 2.16–2.01 (2 s, 6 H, MeCO). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.0–168.3 (Me*CO*), 154.4 (CO), 137.5–127.8 (Ph), 89.4 (C-2, d, $J_{C2,F} = 194.0$ Hz), 77.0 (C-3, d, $J_{C3,F} = 18.6$ Hz), 75.2 (OCH_2Ph, d, $J = 2.6$ Hz), 72.8 (C-4, d, $J_{C4,F} = 8.8$ Hz), 72.2 (C-1, d, $J_{C1,F} = 25.1$ Hz), 66.9 (C-6), 52.5 (C-5), 20.6 (*Me*CO). ^{19}F NMR (282 MHz, $CDCl_3$) δ −198.6. ESIMS: *m/z* 785.21 $[2\ M + Na]^+$. Anal. Calcd for $C_{18}H_{20}FNO_7$: C, 56.69; H, 5.29; F, 4.98; N, 3.67. Found: C, 56.43; H, 5.09; N, 3.45.

(1*R*)-1,4-Di-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-mannonojirimycin (53). Purification by column chromatography (1:3 → 1:1 EtOAc-cyclohexane). Yield: 108 mg (43%). R_f 0.21 (1:1 EtOAc-cyclohexane). $[\alpha]_D$ +3.6 (*c* 1.0 in CH_2Cl_2). ^1H NMR (500 MHz, $CDCl_3$) δ 7.40–7.28 (m, 5 H, Ph), 6.50 (dd, 1 H, $J_{1,F} = 6.1$ Hz, $J_{1,2} = 3.0$ Hz, H-1), 5.19 (t, 1 H, $J_{4,5} = J_{3,4} = 9.6$ Hz, H-4), 4.80 (dt, 1 H, $J_{2,F} = 47.5$ Hz, $J_{2,3} = 2.4$ Hz, H-2), 4.71 (d, 1 H, $^2J_{H,H} = 12.0$ Hz, OCH_2Ph), 4.65 (d, 1 H, OCH_2Ph), 4.41 (dd, 1 H, $J_{6a,6b} = 9.0$ Hz, $J_{5,6a} = 8.2$ Hz, H-6a), 4.33 (dd, 1 H, $J_{5,6b} = 7.2$ Hz, H-6b), 3.90 (bq, 1 H, H-5), 3.78 (ddd, 1 H, $J_{3,F} = 28.2$ Hz, H-3), 2.07–2.05 (2 s, 6 H, MeCO). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.1–168.1 (Me*CO*), 155.1 (CO), 137.0–127.9 (Ph), 85.1 (C-2, d, $J_{C2,F} = 184.8$ Hz), 74.7 (C-1, d, $J_{C1,F} = 20.3$ Hz), 74.5 (C-3, d, $J_{C3,F} = 7.4$ Hz), 72.8 (OCH_2Ph), 70.7 (C-4, d, $J_{C4,F} = 1.68$ Hz), 66.9 (C-6), 53.6 (C-5), 20.7–20.6 (*Me*CO). ^{19}F NMR (282 MHz, $CDCl_3$) δ −202.0. ESIMS: *m/z* 404.15 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{20}FNO_7$: C, 56.69; H, 5.29; N, 3.67. Found: C, 56.41; H, 5.04; N, 3.39.

*4.2.3. Synthesis of the reducing 2-deoxy-2-fluoro-sp^2-iminosugars (**19** , **20** , **21** , **22**)*

General procedure for the deprotection reactions in basic medium.

Over a stirred solution of (1*R*)-1-*O*-acetyl-3,4-di-*O*-benzoyl-2-deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-(manno)nojirimycin (**41** , **42**) or (1*R*)-1,4-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-(manno)nojirimycin (**52** , **53**) (0.20 mmol) in MeOH (6.6 mL), NaOMe (1 M) (0.1 equiv/Bz mol or Ac/mol) was added at room temperature. The reaction mixture was stirred for 60–90 min, diluted with MeOH (3 mL), neutralized with solid CO_2 and concentrated under reduced pressure. The resulting crude was purified by column chromatography using the solvents indicated in each case.

(1*R*)-2-Deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-nojirimycin (19). Compound **19** was obtained from **41** following the general procedure described above. Purification by column chromatography (1:3 → 1:1 EtOAc-cyclohexane → 9:1 EtOAc-MeOH). Yield: 40 mg (97%). R_f 0.56 (9:1 EtOAc-MeOH). $[\alpha]_D$ +43.5 (*c* 1.4 in MeOH). ^1H NMR (300 MHz, CD_3OD) δ 5.48 (d, 1 H, $J_{1,2} = 3.5$ Hz, H-1), 4.54 (t, 1 H, $J_{6a,6b} = J_{5,6a} = 8.6$ Hz, H-6a), 4.24 (ddd, 1 H, $J_{2,F} = 48.7$ Hz, $J_{2,3} = 9.4$ Hz, H-2), 4.21 (dd, 1 H, $J_{5,6b} = 6.9$ Hz, H-6b), 3.96–3.80 (m, 2 H, H-3, H-5), 3.78 (t, 1 H, $J_{4,5} = 9.5$ Hz, H-4). ^{13}C NMR (75.5 MHz, CD_3OD) δ 158.0 (CO), 91.8 (C-2, d, $J_{C2,F} = 189.1$ Hz), 75.3 (C-4, d, $J_{C4,F} = 7.3$ Hz), 74.4 (C-1, d, $J_{C1,F} = 24.8$ Hz), 72.6 (C-3, d, $J_{C3,F} = 17.6$ Hz), 68.5 (C-6), 54.4 (C-5). ^{19}F NMR (282 MHz, CD_3OD) δ −200.9. ESIMS: *m/z* 206.04 $[M - H]^-$. Anal. Calcd for $C_7H_{10}FNO_5$: C, 40.59; H, 4.87; N, 6.76. Found: C, 40.34; H, 4.61; N, 6.42.

(1*R*)-2-Deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-mannonojirimycin (21). Compound **21** was obtained from **42** following the general procedure described above. Purification by column chromatography (1:3 → 1:1 EtOAc-cyclohexane → 9:1 EtOAc-MeOH). Yield: 40 mg (97%). R_f 0.59 (9:1 EtOAc-MeOH). $[\alpha]_D$ +24.7 (*c* 0.9 in MeOH). ^1H NMR (500 MHz, CD_3OD) δ 5.41 (dd, 1 H, $J_{1,F} = 7.4$ Hz, $J_{1,2} = 2.6$ Hz, H-1), 4.73 (dt, 1 H, $J_{2,F} = 49.0$ Hz, $J_{2,3} = 2.4$ Hz, H-2), 4.53 (t, 1 H, $J_{6a,6b} = J_{5,6a} = 8.7$ Hz, H-6a), 4.28 (dd, 1 H, $J_{5,6b} = 5.7$ Hz, H-6b), 3.87–3.80 (m, 1 H, H-5), 3.80 (ddd, 1 H, $J_{3,F} = 30.5$ Hz, $J_{3,4} = 9.7$ Hz, H-3), 3.62 (dt, 1 H, $J_{4,5} = 9.7$ Hz, $J_{4,F} = 1.1$ Hz, H-4). ^{13}C NMR (125.7 MHz, CD_3OD) δ 158.7 (CO), 92.5 (C-2, d, $J_{C2,F} = 177.2$ Hz), 76.4 (C-1, d, $J_{C1,F} = 30.2$ Hz), 72.0 (C-4, d, $J_{C4,F} = 2.5$ Hz), 71.3 (C-3, d, $J_{C3,F} = 17.6$ Hz), 68.2 (C-6), 55.5 (C-5). ^{19}F NMR (282 MHz, $CDCl_3$) δ −204.9. ESIMS: *m/z* 206.02 $[M - H]^-$. Anal. Calcd for $C_7H_{10}FNO_5$: C, 40.59; H, 4.87; N, 6.76. Found: C, 40.45; H, 4.77; N, 6.49.

(1*R*)-3-*O*-Benzyl-2-deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-nojirimycin (20). Compound **20** was obtained from **52** following the general procedure described above. Purification by column chromatography (2:1 EtOAc-cyclohexane). Yield: 58 mg (98%). R_f 0.15 (1:1 EtOAc-cyclohexane). $[\alpha]_D$ +23.3 (*c* 1.0 in MeOH). ^1H NMR (300 MHz, CD_3OD) δ 7.48–7.21 (m, 5 H, Ph), 5.49 (dd, 1 H, $J_{1,2} = 4.0$ Hz, $J_{1,F} = 1.4$ Hz, H-1), 4.82 (bs, 2 H, OCH_2Ph), 4.54 (t, 1 H, $J_{6a,6b} = J_{5,6a} = 8.5$ Hz, H-6a), 4.43 (ddd, 1 H, $J_{2,F} = 48.4$ Hz, $J_{2,3} = 9.3$ Hz, H-2), 4.21 (dd, 1 H, $J_{5,6b} = 6.8$ Hz, H-6b), 3.95–3.80 (m, 2 H, H-5, H-3), 3.52 (t, 1 H, $J_{4,5} = J_{3,4} = 9.4$ Hz, H-4). ^{13}C NMR (75.5 MHz, CD_3OD) δ 157.9 (CO), 139.9–128.6 (Ph), 92.3 (C-2, d, $J_{C2,F} = 190.3$ Hz), 81.0 (C-3, d, $J_{C3,F} = 16.2$ Hz), 76.2 (OCH_2Ph, d, $J = 2.4$ Hz), 75.0 (C-4, d, $J_{C4,F} = 7.7$ Hz), 74.4 (C-1, d, $J_{C1,F} = 24.8$ Hz), 68.5 (C-6), 54.4 (C-5). ^{19}F NMR (282 MHz, CD_3OD) δ −199.0. ESIMS: *m/z* 320.11 $[M + Na]^+$. Anal. Calcd for $C_{14}H_{16}FNO_5$: C, 56.56; H, 5.43; N, 4.71. Found: C, 56.39; H, 5.18; N, 4.40.

(1*R*)-3-*O*-Benzyl-2-deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-mannonojirimycin (22). Compound **22** was obtained from **53** following the general procedure described above. Purification by column chromatography (2:1 EtOAc-cyclohexane → EtOAc). Yield: 56 mg (94%). R_f 0.16 (1:1 EtOAc-cyclohexane). $[\alpha]_D$ −1.7 (*c* 0.8 in MeOH). ^1H NMR (500 MHz, CD_3OD) δ 7.35–7.26 (m, 5 H, Ph), 5.40 (dd, 1 H, $J_{1,F} = 7.2$ Hz, $J_{1,2} = 2.7$ Hz, H-1), 4.86 (ddd, 1 H, $J_{2,F} = 48.8$ Hz, $J_{2,3} = 1.8$ Hz, H-2), 4.74 (bs, 2 H, OCH_2Ph), 4.52 (t, 1 H, $J_{6a,6b} = J_{5,6a} = 8.6$ Hz, H-6a), 4.27 (dd, 1 H, $J_{5,6b} = 5.5$ Hz, H-6b), 3.86 (ddd, 1 H, $J_{4,5} = 9.4$ Hz, H-5),

3.80–3.67 (m, 2 H, H-3, H-4). ^{13}C NMR (125.7 MHz, CD_3OD) δ 158.7 (CO), 139.5–128.5 (Ph), 89.2 (C-2, d, $J_{C2,F}$ = 178.0 Hz), 78.9 (C-3, d, $J_{C3,F}$ = 17.4 Hz), 76.4 (C-1, d, $J_{C1,F}$ = 30.1 Hz), 73.5 (O*C*H_2Ph), 71.3 (C-4, d, $J_{C4,F}$ = 1.8 Hz), 68.2 (C-6), 55.5 (C-5). ^{19}F NMR (470 MHz, CD_3OD) δ −203.8. ESIMS: *m/z* 342.17 $[M + HCO_2]^-$. Anal. Calcd for $C_{14}H_{16}FNO_5$: C, 56.56; H, 5.43; N, 4.71. Found: C, 56.25; H, 5.30; N, 4.46.

*4.2.4. General procedure for the synthesis of pseudo-S-dodecyl glycosides (**43** , **44** , **54** , **55** , **58**)*

To a stirred solution of (1*R*)-1,2,4-tri-*O*-acetyl-3-*O*-benzyl-5*N*,6*O*-oxomethylidenenojirimycin (**40**) or (1*R*)-1-*O*-acetyl-3,4-di-*O*-benzoyl-2-deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-(manno)nojirimycin (**41** , **42**) or (1*R*)-1,4-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-fluoro-5*N*,6*O*-oxomethylidene-(manno)nojirimycin (**52** , **53**) (0.08 mmol) in dry CH_2Cl_2 (1.4 mL), dodecane-1-thiol (39 μL, 0.16 mmol) and BF_3Et_2O (32 μL, 0.27 mmol) were added at 0 °C under Ar atmosphere. The reaction mixture was stirred at room temperature for 1–2 h, diluted with CH_2Cl_2 (50 mL), washed with saturated $NaHCO_3$ (2 × 15 mL), dried ($MgSO_4$), filtered and concentrated. The resulting crude was purified by column chromatography using the solvents indicated in each case.

(1*R*)-3,4-Di-*O*-benzoyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thionojirimycin (43). Compound **43** was obtained from **41** following the general procedure described above. Purification by column chromatography (1:4 EtOAc-cyclohexane). Yield: 35 mg (73%). R_f 0.40 (1:3 EtOAc-cyclohexane). $[\alpha]_D$ +21.5 (*c* 1.3 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98–7.30 (m, 10 H, Ph), 5.97 (q, 1 H, $J_{2,3} = J_{3,4} = J_{3,F}$ = 9.7 Hz, H-3), 5.63 (d, 1 H, $J_{1,2}$ = 5.8 Hz, H-1), 5.26 (t, 1 H, $J_{4,5}$ = 9.4 Hz, H-4), 4.92 (ddd, 1 H, $J_{2,F}$ = 49.4 Hz, H-2), 4.54–4.46 (m, 2 H, H-6a, H-6b), 4.13 (ddd, 1 H, $J_{5,6a}$ = 8.1 Hz, $J_{5,6b}$ = 6.3 Hz, H-5), 2.80–2.58 (m, 2 H, SCH_2), 1.77–1.55 (m, 2 H, SCH_2CH_2), 1.46–1.16 (m, 18 H, CH_2), 0.86 (t, 3 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 165.9–165.3 (CO ester), 155.5 (CO), 133.9–128.1 (Ph), 87.0 (C-2, d, $J_{C2,F}$ = 196.5 Hz), 72.7 (C-4, d, $J_{C4,F}$ = 6.8 Hz), 70.8 (C-3, d, $J_{C3,F}$ = 19.7 Hz), 66.6 (C-6), 58.6 (C-1, d, $J_{C1,F}$ = 25.6 Hz), 52.1 (C-5), 31.9–22.7 (CH_2), 14.1 (CH_3). ^{19}F NMR (470 MHz, $CDCl_3$) δ −190.0. ESIMS: *m/z* 622.44 $[M + Na]^+$. Anal. Calcd for $C_{23}H_{42}FNO_6S$: C, 66.09; H, 7.06; N, 2.34; S, 5.35. Found: C, 66.20; H, 7.19; N, 2.16; S, 5.18.

(1*R*)-3,4-Di-*O*-benzoyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thiomannonojirimycin (44). Compound **44** was obtained from **42** following the general procedure described above. Purification by column chromatography (1:3 EtOAc-cyclohexane). Yield: 27 mg (56%). R_f 0.57 (1:3 EtOAc-cyclohexane). $[\alpha]_D$ −4.7 (*c* 1.0 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97–7.34 (m, 10 H, Ph), 5.75–5.61 (m, 2 H, H-3, H-4), 5.48 (dd, 1 H, $J_{1,F}$ = 13.1 Hz, $J_{1,2}$ = 2.3 Hz, H-1), 5.24 (dt, 1 H, $J_{2,F}$ = 49.6 Hz, $J_{2,3}$ = 2.0 Hz, H-2), 4.53 (d, 2 H, $J_{5,6}$ = 7.3 Hz, H-6), 4.34 (q, 1 H, $J_{4,5}$ = 7.5 Hz, H-5), 2.86–2.65 (m, 2 H, SCH_2), 1.75–1.26 (m, 20 H, CH_2), 0.88 (t, 3 H, $^3J_{H,H}$ = 6.9 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 165.9–169.6 (CO ester), 156.3 (CO), 133.9–128.5 (Ph), 88.8 (C-2, d, $J_{C2,F}$ = 190.9 Hz), 70.6 (C-3, d, $J_{C3,F}$ = 18.0 Hz), 70.2 (C-4, d, $J_{C4,F}$ = 1.8 Hz), 66.6 (C-6), 58.6 (C-1, d, $J_{C1,F}$ = 22.1 Hz), 53.0 (C-5), 32.0–22.8 (CH_2), 14.2 (CH_3). ^{19}F NMR (282 MHz, $CDCl_3$) δ −188.2. ESIMS: *m/z* 622.37 $[M + Na]^+$. Anal. Calcd for $C_{23}H_{20}FNO_8$: C, 66.09; H, 7.06; N, 2.34; S, 5.35. Found: C, 66.22; H, 7.13; N, 2.09; S, 5.14.

(1*R*)-4-*O*-Acetyl-3-*O*-benzyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thionojirimycin (54). Compound **54** was obtained from **52** following the general procedure described above. Purification by column chromatography (1:3 EtOAc-cyclohexane). Yield: 32 mg (77%). R_f 0.61 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ +55.3 (*c* 1.0 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.16 (m, 5 H, Ph), 5.43 (d, 1 H, $J_{1,2}$ = 5.8 Hz, H-1), 4.79 (d, 1 H, $^2J_{H,H}$ = 11.7 Hz, O*C*H_2Ph), 4.77 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 4.66 (ddd, 1 H, $J_{2,F}$ = 47.3 Hz, $J_{2,3}$ = 9.2 Hz, H-2), 4.53 (d, 1 H, O*C*H_2Ph), 4.33 (t, 1 H, $J_{6a,6b} = J_{5,6a}$ = 9.0 Hz, H-6a), 4.20 (dd, 1 H, $J_{5,6b}$ = 7.0 Hz, H-6b), 3.96 (ddd, 1 H, H-5), 3.84 (bq, 1 H, H-3), 2.70–2.46 (m, 2 H, SCH_2), 1.91 (s, 3 H, MeCO), 1.66–1.44 (m, 2 H, SCH_2CH_2), 1.37–1.13 (m, 18 H, CH_2), 0.81 (t, 3 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.1 (Me*C*O), 155.6 (CO), 137.7–127.9 (Ph), 90.0 (C-2, d, $J_{C2,F}$ = 193.3 Hz), 77.8 (C-3, d, $J_{C3,F}$ = 18.0 Hz), 75.2 (O*C*H_2Ph, d, *J* = 2.9 Hz), 73.2 (C-4, d, $J_{C4,F}$ = 8.6 Hz), 66.7 (C-6), 58.6 (C-1, d, $J_{C1,F}$ = 26.4 Hz), 51.8 (C-5), 31.9–22.7 (CH_2), 20.6 (*Me*CO), 14.1 (CH_3). ^{19}F NMR (282 MHz, $CDCl_3$) δ −188.5. ESIMS: *m/z* 546.33 $[M + Na]^+$. Anal. Calcd for $C_{28}H_{42}FNO_5S$: C, 64.22; H, 8.08; N, 2.67; S, 6.12. Found: C, 64.35; H, 8.19; N, 2.40; S, 5.81.

(1*R*)-4-*O*-Acetyl-3-*O*-benzyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thiomannonojirimycin (55). Compound **55** was obtained from **53** following the general procedure described above. Purification by column chromatography (1:4 EtOAc-cyclohexane). Yield: 41 mg (98%). R_f 0.43 (1:3 EtOAc-cyclohexane). $[\alpha]_D$ +23.0 (*c* 1.0 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.26 (m, 5 H, Ph), 5.34 (dd, 1 H, $J_{1,F}$ = 12.9 Hz, $J_{1,2}$ = 2.5 Hz, H-1), 5.16 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 4.88 (dt, 1 H, $J_{2,F}$ = 49.6 Hz, $J_{2,3}$ = 2.2 Hz, H-2), 4.69 (d, 1 H, $^2J_{H,H}$ = 12.0 Hz, O*C*H_2Ph), 4.58 (d, 1 H, O*C*H_2Ph), 4.40 (t, 1 H, $J_{6a,6b} = J_{5,6a}$ = 9.0 Hz, H-6a), 4.32 (dd, 1 H, $J_{5,6b}$ = 6.7 Hz, H-6b), 3.98 (bq, 1 H, H-5), 3.75 (ddd, 1 H, $J_{3,F}$ = 28.4 Hz, H-3), 2.70 (ddd, 1 H, $^2J_{H,H}$ = 12.8 Hz, $^3J_{H,H}$ = 8.0 Hz, $^3J_{H,H}$ = 6.4 Hz, SCH_2), 2.65–2.53 (m, 1 H, SCH_2), 2.02 (s, 3 H, MeCO), 1.70–1.49 (m, 2 H, SCH_2CH_2), 1.40–1.15 (m, 18 H, CH_2), 0.87 (t, 3 H, $^3J_{H,H}$ = 6.9 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.1 (Me*C*O), 156.2 (CO), 137.1–127.9 (Ph), 87.7 (C-2, d, $J_{C2,F}$ = 191.0 Hz), 75.6 (C-3, d, $J_{C3,F}$ = 18.1 Hz), 72.6 (O*C*H_2Ph), 71.0 (C-4, d, $J_{C4,F}$ = 2.3 Hz), 66.6 (C-6), 58.6 (C-1, d, $J_{C1,F}$ = 21.9 Hz), 52.8 (C-5), 32.0 (SCH_2), 31.9–22.7 (CH_2), 20.7 (*Me*CO), 14.1 (CH_3). ^{19}F NMR (282 MHz, $CDCl_3$) δ −188.3. ESIMS: *m/z* 541.29 $[M + NH_4]^+$. Anal. Calcd for $C_{28}H_{42}FNO_5S$: C, 64.22; H, 8.08; N, 2.67; S, 6.12. Found: C, 63.98; H, 7.85; N, 2.39; S, 5.77.

(1*R*)-2,4-Di-*O*-acetyl-3-*O*-benzyl-1-*S*-dodecyl-5*N*,6*O*-oxomethylidene-1-thionojirimycin (58) . Compound **58** was obtained from **40** following the general procedure described above. Purification by column chromatography (1:3 EtOAc-cyclohexane). Yield: 41 mg (92%). R_f 0.48 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ +51.2 (*c* 1.2 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33–7.14 (m, 5 H, Ph), 5.60 (d, 1 H, $J_{1,2}$ = 5.7 Hz, H-1), 4.86 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, H-2), 4.81 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 4.68 (d, 1 H, $^2J_{H,H}$ = 11.7 Hz, O*C*H_2Ph), 4.56 (d, 1 H, O*C*H_2Ph), 4.34 (t, 1 H, $J_{6a,6b} = J_{5,6a}$ = 9.0 Hz, H-6a), 4.23 (dd, 1 H, $J_{5,6b}$ = 7.2 Hz, H-6b), 3.99 (bq, 1 H, H-5), 3.82 (t, 1 H, H-3), 2.56 (ddd, 1 H, $^2J_{H,H}$ = 14.2 Hz, $^3J_{H,H}$ = 8.1 Hz, $^3J_{H,H}$ = 6.2 Hz, SCH_2), 2.41 (ddd, 1 H, SCH_2), 2.01 (2 s, 6 H, MeCO), 1.61–1.10 (m, 20 H, CH_2), 0.81 (t, 3 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.1–169.5 (Me*C*O), 155.5 (CO), 137.9–127.5 (Ph), 77.7 (C-3), 75.6 (O*C*H_2Ph), 74.2 (C-4), 72.5 (C-2), 66.6 (C-6), 58.0 (C-1), 51.8 (C-5), 31.9–22.7 (CH_2), 20.8–20.6 (*Me*CO), 14.1 (CH_3). ESIMS: *m/z* 581.39 $[M + NH_4]^+$. Anal. Calcd for $C_{30}H_{45}NO_7S$: C 63.92, H 8.05, N 2.48, S 5.69. Found: C 64.08, H 8.19, N 2.34, S 5.50.

*4.2.4.1. Synthesis of the unprotected target 2-deoxy-2-fluoro-pseudo-S-glycosides (**10** , **12** , **14** , **16**).*

(1*R*)-2-Deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thionojirimycin (10). Compound **10** was obtained from **43** (40 mg, 0.07 mmol) following the general procedure described above (Section 4.2.3) for the deprotection reaction in basic medium. Purification by column chromatography (1:2 → 1:1 EtOAc-cyclohexane). Yield: 25 mg (95%). R_f 0.58 (2:1 EtOAc-cyclohexane). $[\alpha]_D$ +103.9 (*c* 1.0 in MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 5.44 (d, 1 H, $J_{1,2}$ = 6.3 Hz, H-1), 4.48 (t, 1 H, $J_{6a,6b} = J_{5,6a}$ = 8.7 Hz, H-6a), 4.40 (ddd, 1 H, $J_{2,F}$ = 49.5 Hz, $J_{2,3}$ = 9.4 Hz, H-2), 4.27 (dd, 1 H, $J_{5,6b}$ = 6.0 Hz, H-6b), 3.92 (td, 1 H, $J_{4,5}$ = 9.3 Hz, H-5), 3.75 (dt, 1 H, $J_{3,F}$ = 11.8 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 3.37 (t, 1 H, H-4), 2.72–2.48 (m, 2 H, SCH_2), 1.75–1.25 (m, 20 H, CH_2), 0.90 (t, 3 H, $^3J_{H,H}$ = 6.5 Hz, CH_3). ^{13}C NMR (75.5 MHz, CD_3OD) δ 158.1 (CO), 90.5 (d, $J_{C2,F}$ = 190.6 Hz, C-2), 75.0 (d, $J_{C4,F}$ = 7.2 Hz, C-4), 73.7 (d, $J_{C3,F}$ = 17.1 Hz, C-3), 68.2 (C-6), 59.7 (d, $J_{C1,F}$ = 26.7 Hz, C-1), 54.2 (C-5), 33.0–23.6 (CH_2), 14.3 (CH_3). ^{19}F NMR (282 MHz, CD_3OD) δ −192.2. ESIMS: *m/z* 414.38 $[M + Na]^+$. Anal. Calcd for $C_{19}H_{34}FNO_4S$: C, 58.28; H, 8.75; F, 4.85; N, 3.58; S, 8.19.

Found: C, 58.03; H, 8.61; N, 3.42; S, 7.97.

(1*R*)-2-Deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thiomannonojirimycin (14). Compound **14** was obtained from **44** (61 mg, 0.10 mmol) following the general procedure described above (Section 4.2.3) for the deprotection reaction in basic medium. Purification by column chromatography (1:1 → 3:1 EtOAc-cyclohexane). Yield: 36 mg (90%). R_f 0.22 (1:1 EtOAc-cyclohexane). $[\alpha]_D$ +65.6 (*c* 1.1 in MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.32 (dd, 1 H, $J_{1,F} = 14.5$ Hz, $J_{1,2} = 2.1$ Hz, H-1), 4.89 (dt, 1 H, $J_{2,F} = 49.4$ Hz, $J_{2,3} = 2.1$ Hz, H-2), 4.51 (t, 1 H, $J_{6a,6b} = J_{5,6a} = 9.0$ Hz, H-6a), 4.34 (dd, 1 H, $J_{5,6b} = 4.9$ Hz, H-6b), 3.92 (td, 1 H, $J_{4,5} = 9.0$ Hz, H-5), 3.88-3.70 (m, 3 H, H-3, H-4, OH), 2.69 (ddd, 1 H, $^2J_{H,H} = 14.3$ Hz, $^3J_{H,H} = 8.3$ Hz, $^3J_{H,H} = 6.2$ Hz, SCH_2), 2.62-2.55 (m, 1 H, SCH_2), 1.70-1.53 (m, 2 H, SCH_2CH_2), 1.42-1.21 (m, 18 H, CH_2), 0.87 (t, 3 H, $^3J_{H,H} = 7.0$ Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 157.1 (CO), 91.2 (C-2, d, $J_{C2,F} = 186.0$ Hz), 71.7 (C-3, d, $J_{C3,F} = 18.9$ Hz), 71.0 (C-4, d, $J_{C4,F} = 2.5$ Hz), 66.5 (C-6), 58.8 (C-1, d, $J_{C1,F} = 22.6$ Hz), 53.6 (C-5), 31.9-22.7 (CH_2), 14.1 (CH_3). ^{19}F NMR (470 MHz, $CDCl_3$) δ -189.9. ESIMS: *m/z* 436.34 $[M + HCO_2]^-$. Anal. Calcd for $C_{19}H_{34}FNO_4S$: C, 58.28; H, 8.75; N, 3.58; S, 8.19. Found: C, 58.19; H, 8.61; N, 3.48; S, 7.93.

(1*R*)-3-*O*-Benzyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thionojirimycin (12). Compound **12** was obtained from **54** (25 mg, 0.05 mmol) following the general procedure described above (Section 4.2.3) for the deprotection reaction in basic medium. Purification by column chromatography (1:3 EtOAc-cyclohexane). Yield: 22 mg (95%). R_f 0.33 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ +56.1 (*c* 0.9 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45-7.29 (m, 5 H, Ph), 5.48 (d, 1 H, $J_{1,2} = 6.0$ Hz, H-1), 4.97 (d, 1 H, $^2J_{H,H} = 11.3$ Hz, OCH_2Ph), 4.67 (ddd, 1 H, $J_{2,F} = 47.9$ Hz, $J_{2,3} = 9.3$ Hz, H-2), 4.60 (d, 1 H, OCH_2Ph), 4.50 (t, 1 H, $J_{6a,6b} = J_{5,6a} = 9.0$ Hz, H-6a), 4.23 (dd, 1 H, $J_{5,6b} = 5.5$ Hz, H-6b), 3.95 (ddd, 1 H, $J_{4,5} = 9.5$ Hz, H-5), 3.76 (q, 1 H, $J_{3,4} = J_{3,F} = 9.3$ Hz, H-3), 3.41 (t, 1 H, H-4), 2.74-2.52 (m, 2 H, SCH_2), 1.73-1.19 (m, 20 H, CH_2), 0.88 (t, 3 H, $^3J_{H,H} = 7.0$ Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 155.8 (CO), 137.6-128.2 (Ph), 90.3 (C-2, d, $J_{C2,F} = 193.3$ Hz), 80.2 (C-3, d, $J_{C3,F} = 15.9$ Hz), 75.3 (OCH_2Ph, d, $J = 4.5$ Hz), 73.2 (C-4, d, $J_{C4,F} = 7.6$ Hz), 66.5 (C-6), 58.9 (C-1, d, $J_{C1,F} = 26.4$ Hz), 52.1 (C-5), 31.9-22.7 (CH_2), 14.1 (CH_3). ^{19}F NMR (282 MHz, $CDCl_3$) δ -188.6. ESIMS: *m/z* 526.36 $[M + HCO_2]^-$. Anal. Calcd for $C_{26}H_{40}FNO_4S$: C, 64.83; H, 8.37; N, 2.91; S, 6.66. Found: C, 64.63; H, 8.16; N, 2.66; S, 6.42.

(1*R*)-3-*O*-Benzyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thiomannonojirimycin (16). Compound **16** was obtained from **55** (43 mg, 0.08 mmol) following the general procedure described above (Section 4.2.3) for the deprotection reaction in basic medium. Purification by column chromatography (1:4 EtOAc-cyclohexane). Yield: 36 mg (91%). R_f 0.45 (1:3 EtOAc-cyclohexane). $[\alpha]_D$ +43.1 (*c* 0.9 in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41-7.32 (m, 5 H, Ph), 5.35 (dd, 1 H, $J_{1,F} = 13.8$ Hz, $J_{1,2} = 2.3$ Hz, H-1), 4.91 (dt, 1 H, $J_{2,F} = 49.5$ Hz, $J_{2,3} = 2.1$ Hz, H-2), 4.75 (d, 1 H, $^2J_{H,H} = 11.5$ Hz, OCH_2Ph), 4.57 (d, 1 H, OCH_2Ph), 4.51 (dd, 1 H, $J_{6a,6b} = 9.0$ Hz, $J_{5,6a} = 8.2$ Hz, H-6a), 4.28 (dd, 1 H, $J_{5,6b} = 5.3$ Hz, H-6b), 4.21 (td, 1 H, $J_{4,5} = 9.0$ Hz, H-5), 3.86 (t, 1 H, $J_{3,4} = 9.4$ Hz, H-4), 3.59 (ddd, 1 H, $J_{3,F} = 28.2$ Hz, H-3), 2.71 (ddd, 1 H, $^2J_{H,H} = 12.6$ Hz, $^3J_{H,H} = 8.2$ Hz, $^3J_{H,H} = 6.1$ Hz, SCH_2), 2.62-2.55 (m, 2 H, SCH_2, OH), 1.70-1.53 (m, 2 H, SCH_2CH_2), 1.41-1.21 (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 156.6 (CO), 136.9-128.2 (Ph), 87.0 (C-2, d, $J_{C2,F} = 189.8$ Hz), 78.7 (C-3, d, $J_{C3,F} = 18.9$ Hz), 72.2 (OCH_2Ph), 69.9 (C-4, d, $J_{C4,F} = 3.8$ Hz), 66.5 (C-6), 58.9 (C-1, d, $J_{C1,F} = 22.6$ Hz), 53.3 (C-5), 31.9-22.7 (CH_2), 14.1 (CH_3). ^{19}F NMR (470 MHz, $CDCl_3$) δ -188.4. ESIMS: *m/z* 526.40 $[M + HCO_2]^-$. Anal. Calcd for $C_{26}H_{40}FNO_4S$: C, 64.83; H, 8.37; N, 2.91; S, 6.66. Found: C, 64.59; H, 8.12; N, 2.60; S, 6.33.

*4.2.5. General procedure for the acetylation reactions of 2-deoxy-2-fluoro-pseudo-S-glycosides (**48** , **49**)*

Over a solution of (1*R*)-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thio(manno)nojirimycin (**10** or **14**) (0.16 mmol) in pyridine (0.5 mL), Ac_2O (0.5 mL) was added at 0 °C and the reaction mixture was stirred at room temperature for 2 h 30 min, diluted with CH_2Cl_2 and ice-water, washed with 1N HCl (3 × 25 mL), saturated $NaHCO_3$ (3 × 25 mL) and water (2 × 10 mL), dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified by column chromatography using the solvents indicated in each case.

(1*R*)-3,4-Di-*O*-acetyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thionojirimycin (48). Compound **48** was obtained from **10** following the general procedure described above. Purification by column chromatography (1:1 EtOAc-cyclohexane). Yield: 72 mg (96%). R_f 0.64 (2:1 EtOAc-cyclohexane). $[\alpha]_D$ +88.3 (*c* 1.0 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.51 (d, 1 H, $J_{1,2} = 5.7$ Hz, H-1), 5.48 (q, 1 H, $J_{2,3} = J_{3,4} = J_{3,F} = 9.5$ Hz, H-3), 4.88 (t, 1 H, $J_{4,5} = 9.6$ Hz, H-4), 4.69 (ddd, 1 H, $J_{2,F} = 49.2$ Hz, H-2), 4.45 (dd, 1 H, $J_{6a,6b} = 9.0$ Hz, $J_{5,6a} = 8.0$ Hz, H-6a), 4.26 (dd, 1 H, $J_{5,6b} = 6.3$ Hz, H-6b), 4.14 (ddd, 1 H, H-5), 2.76–2.52 (m, 2 H, SCH_2), 2.07 (2 s, 6 H, MeCO), 1.70–1.18 (m, 20 H, CH_2), 0.87 (t, 3 H, $J_{H,H} = 7.0$ Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.0–169.5 (Me*CO*), 155.3 (CO), 86.8 (C-2, d, $J_{C2,F} = 196.2$ Hz), 72.1 (C-4, d, $J_{C4,F} = 6.7$ Hz), 70.5 (C-3, d, $J_{C3,F} = 19.6$ Hz), 66.3 (C-6), 58.3 (C-1, d, $J_{C1,F} = 25.8$ Hz), 51.5 (C-5), 31.9–22.7 (CH_2), 20.6–20.5 (*Me*CO), 14.1 (CH_3). ^{19}F NMR (282 MHz, $CDCl_3$) δ −190.3. ESIMS: *m/z* 498.37 $[M + Na]^+$. Anal. Calcd for $C_{23}H_{38}FNO_6S$: C, 58.08; H, 8.05; N, 2.95; S, 6.74. Found: C, 58.14; H, 8.11; N, 2.86; S, 6.52.

(1*R*)-3,4-Di-*O*-acetyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thiomannonojirimycin (49). Compound **49** was obtained from **14** following the general procedure described above. Purification by column chromatography (1:3 EtOAc-cyclohexane). Yield: 60 mg (79%). R_f 0.50 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ +30.0 (*c* 1.0 in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (dd, 1 H, $J_{1,F} = 13.2$ Hz, $J_{1,2} = 2.5$ Hz, H-1), 5.28–5.17 (m, 2 H, H-3, H-4), 4.94 (dt, 1 H, $J_{2,F} = 49.4$ Hz, H-2), 4.45 (dd, 1 H, $J_{6a,6b} = 9.2$ Hz, $J_{5,6a} = 8.5$ Hz, H-6a), 4.34 (dd, 1 H, $J_{5,6b} = 6.3$ Hz, H-6b), 4.14–4.08 (m, 1 H, H-5), 2.78–2.60 (m, 2 H, SCH_2), 2.10–2.07 (2 s, 6 H, MeCO), 1.70–1.24 (m, 20 H, CH_2), 0.87 (t, 3 H, $J_{H,H} = 7.1$ Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.1–169.9 (Me*CO*), 156.2 (CO), 88.5 (C-2, d, $J_{C2,F} = 190.4$ Hz), 70.0 (C-3, d, $J_{C3,F} = 17.8$ Hz), 69.7 (C-4, d, $J_{C4,F} = 1.5$ Hz), 66.5 (C-6), 58.4 (C-1, d, $J_{C1,F} = 22.2$ Hz), 52.7 (C-5), 32.0–22.8 (CH_2), 20.8–20.7 (*Me*CO), 14.2 (CH_3). ^{19}F NMR (282 MHz, $CDCl_3$) δ −188.4. ESIMS: *m/z* 498.34 $[M + Na]^+$. Anal. Calcd for $C_{23}H_{38}FNO_6S$: C, 58.08; H, 8.05; N, 2.95; S, 6.74. Found: C, 58.33; H, 8.26; N, 2.74; S, 6.47.

*4.2.6. General procedure for the synthesis of dodecylsulfonyl-sp^2-IGLs (**45** , **46** , **50** , **51** , **56** , **57** , **59**)*

To a stirred solution of (1*R*)-3,4-di-*O*-benzoyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thio(manno)nojirimycin (**43** , **44**) or (1*R*)-3,4-di-*O*-acetyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thio(manno)nojirimycin (**48** , **49**) or (1*R*)-4-*O*-acetyl-3-*O*-benzyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-thio (manno)nojirimycin (**54** , **55**) or (1*R*)-2,4-di-*O*-acetyl-3-*O*-benzyl-1-*S*-dodecyl-5*N*,6*O*-oxomethylidene-1-thionojirimycin (**58**) (0.14 mmol) in CH_2Cl_2 (3 mL), MCPBA (70 mg, 0.28 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 60–90 min, diluted with CH_2Cl_2 (50 mL), washed with saturated $NaHCO_3$ (5 × 15 mL), dried ($MgSO_4$), filtered and concentrated. The resulting crude was purified by column chromatography using the solvents indicated in each case.

(1*R*)-3,4-Di-*O*-benzoyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonylnojirimycin (45). Compound **45** was obtained from **43** following the general procedure described above. Purification by column chromatography (1:2 EtOAc-cyclohexane). Yield: 84 mg (95%). R_f 0.46 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ −34.4 (*c* 1.1 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05–7.31 (m, 10 H, Ph), 6.44 (q, 1 H, $J_{2,3} = J_{3,4} = J_{3,F} = 8.1$ Hz, H-3), 5.40 (ddd, 1 H, $J_{2,F} = 48.2$ Hz, $J_{1,2} = 6.2$ Hz, H-2), 5.44–5.35 (m, 2 H, H-1, H-4), 4.70–4.53 (m, 3 H, H-6a, H-6b, H-5), 3.23 (t, 2 H, $J = 8.0$ Hz, SO_2CH_2), 2.06–1.81 (m, 2 H, $SO_2CH_2CH_2$), 1.52–1.17 (m, 18 H, CH_2), 0.86 (t, 3 H, $^3J_{H,H} = 6.4$ Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 165.8–164.8 (CO ester), 156.0 (CO), 134.5–127.4 (Ph), 86.1 (C-2, d, $J_{C2,F} = 191.9$ Hz), 72.2 (C-4, d, $J_{C4,}$

$_F$ = 5.5 Hz), 69.5 (C-3, d, $J_{C3,F}$ = 22.4 Hz), 67.5 (C-6), 67.3 (C-1, d, $J_{C1,F}$ = 21.1 Hz), 54.7 (SO_2CH_2), 53.8 (C-5), 31.9–21.3 (CH_2), 14.1 (CH_3). ^{19}F NMR (470 MHz, $CDCl_3$) δ −199.4. ESIMS: *m/z* 654.36 $[M + Na]^+$. Anal. Calcd for $C_{33}H_{42}FNO_8S$: C, 62.74; H, 6.70; F, 3.01; N, 2.22; S, 5.07. Found: C, 62.91; H, 6.83; N, 1.97; S, 4.85.

(1*R*)-3,4-Di-*O*-benzoyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonylmannonojirimycin (46). Compound **46** was obtained from **44** following the general procedure described above. Purification by column chromatography (1:6 → 1:4 EtOAc-cyclohexane). Yield: 87 mg (98%). R_f 0.40 (1:3 EtOAc-cyclohexane). $[\alpha]_D$ −46.7 (*c* 1.1 in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00–7.34 (m, 10 H, Ph), 5.93 (ddd, 1 H, $J_{3,F}$ = 28.4 Hz, $J_{3,4}$ = 10.0 Hz, $J_{2,3}$ = 2.3 Hz, H-3), 5.80 (dt, 1 H, $J_{2,F}$ = 47.6 Hz, $J_{1,2}$ = 2.2 Hz, H-2), 5.69 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 5.39 (dd, 1 H, $J_{1,F}$ = 16.0 Hz, H-1), 4.69–4.58 (m, 3 H, H-5, H-6a, H-6b), 3.26–3.08 (m, 2 H, SO_2CH_2), 1.94–1.85 (m, 2 H, $SO_2CH_2CH_2$), 1.50–1.26 (m, 18 H, CH_2), 0.88 (t, 3 H, $^3J_{H,H}$ = 7.1 Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 166.0–165.2 (CO ester), 156.4 (CO), 134.1–128.2 (Ph), 83.9 (C-2, d, $J_{C2,F}$ = 190.2 Hz), 70.4 (C-3, d, $J_{C3,F}$ = 17.7 Hz), 69.9 (C-1, d, $J_{C1,F}$ = 22.9 Hz), 69.1 (C-4, d, $J_{C4,F}$ = 2.8 Hz), 67.4 (C-6), 54.4 (C-5), 52.5 (SO_2CH_2), 32.0–21.7 (CH_2), 14.2 (CH_3). ^{19}F NMR (470 MHz, $CDCl_3$) δ −201.2. ESIMS: *m/z* 649.39 $[M + NH_4]^+$. Anal. Calcd for $C_{33}H_{42}FNO_8S$: C, 62.74; H, 6.70; N, 2.22; S, 5.07. Found: C, 62.39; H, 6.38; N, 1.87; S, 4.74.

(1*R*)-3,4-Di-*O*-acetyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonylnojirimycin (50). Compound **50** was obtained from **48** following the general procedure described above. Purification by column chromatography (1:2 EtOAc-cyclohexane). Yield: 67 mg (95%). R_f 0.52 (1:1 EtOAc-cyclohexane). $[\alpha]_D$ +11.5 (*c* 1.0 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.93 (dt, 1 H, $J_{3,F}$ = 9.5 Hz, $J_{3,4}$ = $J_{2,3}$ = 8.4 Hz, H-3), 5.19 (d, 1 H, $J_{1,2}$ = 6.2 Hz, H-1), 5.10 (ddd, 1 H, $J_{2,F}$ = 46.7 Hz, H-2), 4.92 (t, 1 H, $J_{4,5}$ = 8.1 Hz, H-4), 4.61–4.50 (m, 1 H, H-6a), 4.41–4.29 (m, 2 H, H-5, H-6b), 3.15 (t, 2 H, $^3J_{H,H}$ = 8.0 Hz, SO_2CH_2), 2.08–2.07 (2s, 6 H, MeCO), 1.99–1.78 (m, 2 H, $SO_2CH_2CH_2$), 1.51–1.108 (m, 18 H, CH_2), 0.87 (t, 3 H, $^3J_{H,H}$ = 6.9 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.1–169.1 (Me*CO*), 155.7 (CO), 86.0 (C-2, d, $J_{C2,F}$ = 192.0 Hz), 71.7 (C-4, d, $J_{C4,F}$ = 5.6 Hz), 69.3 (C-3, d, $J_{C3,F}$ = 22.3 Hz), 67.1 (C-6), 67.0 (C-1, d, $J_{C1,F}$ = 25.0 Hz), 54.7 (*J* = 4.5 Hz, SO_2CH_2), 53.2 (C-5), 31.9–21.3 (CH_2), 20.5 (*Me*CO), 14.1 (CH_3). ^{19}F NMR (282 MHz, $CDCl_3$) δ −199.4. ESIMS: *m/z* 525.43 $[M + NH_4]^+$. Anal. Calcd for $C_{23}H_{38}FNO_8S$: C, 54.42; H, 7.55; F, 3.74; N, 2.76; S, 6.32. Found: C, 54.48; H, 7.47; N, 2.61; S, 6.15.

(1*R*)-3,4-Di-*O*-acetyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonylmannonojirimycin (51). Compound **51** was obtained from **49** following the general procedure described above. Purification by column chromatography (1:2 EtOAc-cyclohexane). Yield: 68 mg (96%). R_f 0.39 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ −12.3 (*c* 0.8 in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.58 (dt, 1 H, $J_{2,F}$ = 47.5 Hz, $J_{1,2}$ = $J_{2,3}$ = 2.3 Hz, H-2), 5.47 (ddd, 1 H, $J_{3,F}$ = 28.3 Hz, $J_{3,4}$ = 9.9 Hz, H-3), 5.27 (dd, 1 H, $J_{1,F}$ = 15.9 Hz, H-1), 5.24 (t, 1 H, $J_{4,5}$ = 9.1 Hz, H-4), 4.56–4.52 (m, 1 H, H-6a), 4.44–4.39 (m, 2 H, H-5, H-6b), 3.17–3.03 (m, 2 H, SO_2CH_2), 2.11–2.08 (2s, 6 H, MeCO), 1.98–1.80 (m, 2 H, $SO_2CH_2CH_2$), 1.47–1.24 (m, 18 H, CH_2), 0.87 (t, 3 H, $^3J_{H,H}$ = 7.1 Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.2–169.4 (Me*CO*), 156.3 (CO), 83.7 (C-2, d, $J_{C2,F}$ = 189.9 Hz), 69.8 (C-3, d, $J_{C3,F}$ = 17.9 Hz), 69.7 (C-1, d, $J_{C1,F}$ = 23.1 Hz), 68.5 (C-4, d, $J_{C4,F}$ = 2.8 Hz), 67.3 (C-6), 54.0 (C-5), 52.4 (SO_2CH_2), 32.0–21.6 (CH_2), 20.6 (*Me*CO), 14.2 (CH_3). ^{19}F NMR (470 MHz, $CDCl_3$) δ −201.3. ESIMS: *m/z* 525.34 $[M + NH_4]^+$. Anal. Calcd for $C_{23}H_{38}FNO_8S$: C, 54.42; H, 7.55; N, 2.76; S, 6.32. Found: C, 54.21; H, 7.36; N, 2.53; S, 6.01.

(1*R*)-4-*O*-Acetyl-3-*O*-benzyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonylnojirimycin (56). Compound **56** was obtained from **54** following the general procedure described above. Purification by column chromatography (1:2 EtOAc-cyclohexane). Yield: 62 mg (80%). R_f 0.30 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ −4.2 (*c* 1.2 in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.26 (m, 5 H, Ph), 5.21 (dd, 1 H, $J_{1,F}$ = 8.6 Hz, $J_{1,2}$ = 6.0 Hz, H-1), 5.12 (ddd, 1 H, $J_{2,F}$ = 46.9 Hz, $J_{2,3}$ = 7.6 Hz, H-2), 4.90 (dd, 1 H, $J_{4,5}$ = 9.0 Hz, $J_{3,4}$ = 8.0 Hz, H-4), 4.80 (d, 1 H, $^2J_{H,H}$ = 11.8 Hz, OCH_2Ph), 4.68 (d, 1 H, OCH_2Ph), 4.51 (dd, 1 H, $J_{6a,6b}$ = 9.0 Hz, $J_{5,6a}$ = 8.0 Hz, H-6a), 4.45 (dt, 1 H, $J_{3,F}$ = 9.1 Hz, H-3), 4.34 (dd, 1 H, $J_{5,6b}$ = 5.3 Hz, H-6b), 4.25 (td, 1 H, H-5), 3.19–3.12 (m, 2 H, SO_2CH_2), 1.99 (s, 3 H, MeCO), 1.97–1.80 (m, 2 H, $SO_2CH_2CH_2$), 1.49–1.20 (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 170.2 (Me*CO*), 155.9 (CO), 137.2–127.9 (Ph), 88.3 (C-2, d, $J_{C2,F}$ = 189.8 Hz), 76.1 (C-3, d, $J_{C3,F}$ = 21.4 Hz), 74.6 (OCH_2Ph), 72.5 (C-4, d, $J_{C4,F}$ = 6.3 Hz), 67.5 (C-6), 67.3 (C-1, d, $J_{C1,F}$ = 24.2 Hz), 54.6 (*J* = 3.6 Hz, SO_2CH_2), 53.5 (C-5), 31.9–21.4 (CH_2), 20.6 (*Me*CO), 14.1 (CH_3). ^{19}F NMR (470 MHz, $CDCl_3$) δ −198.6. ESIMS: *m/z* 600.41 $[M + HCO_2]^-$. Anal. Calcd for $C_{28}H_{42}FNO_7S$: C, 60.52; H, 7.62; N, 2.52; S, 5.77. Found: C, 60.34; H, 7.47; N, 2.31; S, 5.49.

(1*R*)-4-*O*-Acetyl-3-*O*-benzyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonylmannonojirimycin (57). Compound **57** was obtained from **55** following the general procedure described above. Purification by column chromatography (1:2 EtOAc-cyclohexane). Yield: 73 mg (94%). R_f 0.55 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ −27.7 (*c* 1.0 in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40–7.29 (m, 5 H, Ph), 5.51 (dt, 1 H, $J_{2,F}$ = 47.3 Hz, $J_{2,3}$ = $J_{1,2}$ = 2.2 Hz, H-2), 5.26 (dd, 1 H, $J_{1,F}$ = 15.5 Hz, H-1), 5.18 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 4.76 (d, 1 H, $^2J_{H,H}$ = 11.9 Hz, OCH_2Ph), 4.64 (d, 1 H, OCH_2Ph), 4.51 (t, 1 H, $J_{6a,6b}$ = $J_{5,6a}$ = 9.0 Hz, H-6a), 4.41 (dd, 1 H, $J_{5,6b}$ = 6.4 Hz, H-6b), 4.30 (td, 1 H, H-5), 4.06 (ddd, 1 H, $J_{3,F}$ = 28.1 Hz, H-3), 3.13 (ddd, 1 H, $^2J_{H,H}$ = 14.2 Hz, $^3J_{H,H}$ = 10.4 Hz, $^3J_{H,H}$ = 5.6 Hz, SO_2CH_2), 3.04 (ddd, 1 H, SO_2CH_2), 2.03 (s, 3 H, MeCO), 1.96–1.77 (m, 2 H, $SO_2CH_2CH_2$), 1.47–1.21 (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 170.2 (Me*CO*), 156.3 (CO), 136.8–128.0 (Ph), 82.9 (C-2, d, $J_{C2,F}$ = 189.8 Hz), 75.9 (C-3, d, $J_{C3,F}$ = 17.6 Hz), 73.1 (OCH_2Ph), 70.0 (C-4, d, $J_{C4,F}$ = 3.7 Hz), 69.8 (C-1, d, $J_{C1,F}$ = 23.4 Hz), 67.6 (C-6), 54.3 (C-5), 52.5 (SO_2CH_2), 31.9–21.5 (CH_2), 20.7 (*Me*CO), 14.1 (CH_3). ^{19}F NMR (470 MHz, $CDCl_3$) δ −201.8. ESIMS: *m/z* 573.29 $[M + Na]^+$. Anal. Calcd for $C_{28}H_{42}FNO_7S$: C, 60.52; H, 7.62; N, 2.52; S, 5.77. Found: C, 60.23; H, 7.46; N, 2.27; S, 5.51.

(1*R*)-2,4-Di-*O*-acetyl-3-*O*-benzyl-1-*S*-dodecyl-5*N*,6*O*-oxomethylidene-1-sulfonylnojirimycin (59) . Compound **59** was obtained from **58** following the general procedure described above. Purification by column chromatography (1:4 EtOAc-cyclohexane). Yield: 47 mg (56%). R_f 0.46 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ +17.5 (*c* 1.2 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33–7.15 (m, 5 H, Ph), 5.35 (d, 1 H, $J_{1,2}$ = 6.4 Hz, H-1), 5.17 (dd, 1 H, $J_{2,3}$ = 9.0 Hz, H-2), 4.84 (dd, 1 H, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 8.0 Hz, H-4), 4.72 (d, 1 H, $^2J_{H,H}$ = 11.8 Hz, OCH_2Ph), 4.63 (d, 1 H, OCH_2Ph), 4.43 (t, 1 H, $J_{6a,6b}$ = $J_{5,6a}$ = 9.0 Hz, H-6a), 4.39–4.29 (m, 2 H, H-3, H-6b), 4.23 (td, 1 H, $J_{5,6b}$ = 5.0 Hz, H-5), 3.02–2.82 (m, 2 H, SO_2CH_2), 2.04–1.90 (2 s, 6 H, MeCO), 1.87–1.64 (m, 2 H, $SO_2CH_2CH_2$), 1.38–1.10 (m, 18 H, CH_2), 0.81 (t, 3 H, $^3J_{H,H}$ = 6.9 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.1 (Me*CO*), 155.6 (CO), 137.5–127.6 (Ph), 76.3 (C-3), 75.3 (OCH_2Ph), 73.1 (C-4), 70.0 (C-2), 67.3 (C-6), 65.9 (C-1), 53.2 (C-5), 52.9 (SO_2CH_2), 31.9–21.2 (CH_2), 20.7–20.6 (*Me*CO), 14.1 (CH_3). ESIMS: *m/z* 613.38 $[M + Na]^+$. Anal. Calcd for $C_{30}H_{45}NO_9S$: C 60.48, H 7.61, N 2.35, S 5.38. Found: C 60.61, H 7.72, N 2.24, S 5.13.

*4.2.7. Deprotection reactions of sulfonyl-sp^2-IGLs in basic medium (**18** , **47**)*

(1*R*)-3-*O*-Benzyl-1-*S*-dodecyl-5*N*,6*O*-oxomethylidene-1-sulfonylnojirimycin (18) . Compound **18** was obtained by conventional de-*O*-acetylation of **59** (111 mg, 0.19 mmol) followed by purification by column chromatography (1:3 → 1:1 EtOAc-cyclohexane). Yield: 87 mg (90%). R_f 0.36 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ +11.3 (*c* 1.3 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.28 (m, 5 H, Ph), 5.11 (d, 1 H, $J_{1,2}$ = 5.4 Hz, H-1), 4.97 (d, 1 H, $^2J_{H,H}$ = 11.6 Hz, OCH_2Ph), 4.77 (d, 1 H, OCH_2Ph), 4.57 (t, 1 H, $J_{6a,6b}$ = $J_{5,6a}$ = 8.4 Hz, H-6a), 4.30–4.06 (m, 4 H, H-2, H-4, H-5, H-6b), 3.43 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 8.5 Hz, H-3), 3.27–3.07 (m, 2 H, SO_2CH_2), 1.98–1.75 (m, 2 H, $SO_2CH_2CH_2$), 1.50–1.19 (m, 18 H, CH_2), 0.88 (t, 3 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ

156.4 (CO), 137.8–128.1 (Ph), 81.1 (C-4), 75.5 (OCH_2Ph), 73.7 (C-3), 71.3 (C-2), 69.1 (C-1), 67.4 (C-6), 54.9 (CH_2SO_2), 54.7 (C-5), 31.9–21.3 (CH_2), 14.1 (CH_3). ESIMS: *m/z* 529.44 $[M + NH_4]^+$. Anal. Calcd for $C_{26}H_{41}NO_7S$: C 61.03, H 8.08, N 2.74, S 6.27. Found: C 60.86, H 7.87, N 2.55, S 6.02.

(1*R*)-1-*S*-Dodecyl-2-metoxy-5*N*,6*O*-oxomethylidene-1-sulfonylmannonojirimycin (47). Over a stirred solution of (1*R*)-3,4-di-*O*-benzoyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonylnojirimycin (**45**) (70 mg, 0.11 mmol) in MeOH (3 mL), NaOMe (1 M) (203 μL, 0.20 mmol, 1.8 eq.) was added in certain portions at room temperature and the reaction mixture was stirred for 3 h, diluted with MeOH (3 mL) and neutralized with solid CO_2. The solvent was removed under reduced pressure and the resulting crude was purified by column chromatography (1:1 EtOAc-cyclohexane). Yield: 17 mg (36%). R_f 0.29 (2:1 EtOAc-cyclohexane). $[\alpha]_D$ −4.3 (*c* 1.2 in MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 5.21 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 4.60 (t, 1 H, $J_{6a,6b}$ = $J_{5,6a}$ = 9.0 Hz, H-6a), 4.35 (dd, $J_{5,6b}$ = 4.5 Hz, H-6b), 4.20 (dd, 1 H, $J_{2,3}$ = 3.1 Hz, H-2), 4.06 (ddd, 1 H, $J_{4,5}$ = 9.5 Hz, H-5), 3.91 (dd, 1 H, $J_{3,4}$ = 9.5 Hz, H-3), 3.66 (t, 1 H, H-4), 3.45 (s, 3 H, OMe), 3.29–3.12 (m, 2 H, SO_2CH_2), 1.93–1.70 (m, 2 H, $SO_2CH_2CH_2$), 1.53–1.21 (m, 18 H, CH_2), 0.90 (t, 3 H, $^3J_{H,H}$ = 6.5 Hz, CH_3). ^{13}C NMR (75.5 MHz, CD_3OD) δ 158.1 (CO), 76.0 (C-2), 71.5 (C-3), 69.3 (C-4), 68.6 (C-1), 67.1 (C-6), 57.6 (OMe), 55.6 (C-5), 50.7 (SO_2CH_2), 31.7–21.1 (CH_2), 13.0 (CH_3). ESIMS: *m/z* 458.33 $[M + Na]^+$. Anal. Calcd for $C_{20}H_{37}NO_7S$: C, 55.15; H, 8.56; N, 3.22; S, 7.36. Found: C, 54.88; H, 8.25; N, 2.96; S, 7.13.

*4.2.8. General procedure for the deprotection reaction of 2-deoxy-2-fluoro-sulfonyl-sp^2-IGLs in acid medium (**11** , **13** , **15** , **17**)*

To a stirred solution of (1*R*)-3,4-di-*O*-acetyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonyl(manno)nojirimycin (**50** , **51**) or (1*R*)-4-*O*-acetyl-3-*O*-benzyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonyl(manno)nojirimycin (**56** , **57**) (0.05 mmol) in CH_3CN (0.5 mL), a solution of HCl in MeOH (7%) (1.8 mL) was added and the reaction mixture was stirred at room temperature for 24 h. Then, the solvent was removed under reduced pressure and the residue was dissolved in EtOAc (20 mL), washed with saturated $NaHCO_3$ (2 × 15 mL) and H_2O (15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure and the resulting crude was purified by column chromatography using the solvents indicated in each case.

(1*R*)-2-Deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonylnojirimycin (11). Compound **11** was obtained from **50** following the general procedure described above. Purification by column chromatography (3:1 EtOAc-cyclohexane). Yield: 20 mg (92%). R_f 0.25 (2:1 EtOAc-cyclohexane). $[\alpha]_D$ +18.3 (*c* 1.1 in MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 5.48 (d, 1 H, $J_{1,2}$ = 6.8 Hz, H-1), 4.84 (ddd, 1 H, $J_{2,F}$ = 47.3 Hz, $J_{2,3}$ = 9.4 Hz, H-2), 4.65 (t, 1 H, $J_{6a,6b}$ = $J_{5,6a}$ = 8.7 Hz, H-6a), 4.32–4.16 (m, 2 H, H-3, H-6b), 4.04 (ddd, 1 H, $J_{4,5}$ = 9.4 Hz, $J_{5,6b}$ = 6.1 Hz, H-5), 3.41 (t, 1 H, $J_{3,4}$ = 9.4 Hz, H-4), 3.20–3.03 (m, 2 H, SO_2CH_2), 1.86 (quint., 2 H, $^3J_{H,H}$ = 7.5 Hz, $SO_2CH_2CH_2$), 1.55–1.20 (m, 18 H, CH_2), 0.90 (t, 3 H, $^3J_{H,H}$ = 6.5 Hz, CH_3). ^{13}C NMR (75.5 MHz, CD_3OD) δ 156.6 (CO), 89.5 (C-2, d, $J_{C2,F}$ = 188.8 Hz), 73.3 (C-4, d, $J_{C4,F}$ = 7.6 Hz), 71.2 (C-3, d, $J_{C3,F}$ = 17.4 Hz), 68.0 (C-1, d, $J_{C1,F}$ = 26.4 Hz), 67.3 (C-6), 54.6 (C-5), 53.7 (d, *J* = 5.0 Hz, SO_2CH_2), 31.7–21.0 (CH_2), 13.0 (CH_3). ^{19}F NMR (282 MHz, CD_3OD) δ −200.99. ESIMS: *m/z* 468.48 $[M + HCO_2]^-$. Anal. Calcd for $C_{19}H_{34}FNO_6S$: C, 53.88; H, 8.09; N, 3.31; S, 7.57. Found: C, 53.63; H, 7.88; N, 3.06; S, 7.34.

(1*R*)-2-Deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonylmannonojirimycin (15). Compound **15** was obtained from **51** following the general procedure described above. Purification by column chromatography (3:1 EtOAc-cyclohexane). Yield: 17 mg (82%). R_f 0.45 (2:1 EtOAc-cyclohexane). $[\alpha]_D$ +7.7 (*c* 1.0 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ 5.44 (dd, 1 H, $J_{1,F}$ = 16.8 Hz, $J_{1,2}$ = 1.7 Hz, H-1), 5.39 (dt, 1 H, $J_{2,F}$ = 47.2 Hz, $J_{2,3}$ = 2.1 Hz, H-2), 4.64 (t, 1 H, $J_{6a,6b}$ = $J_{5,6a}$ = 8.8 Hz, H-6a), 4.39 (m, 1 H, $J_{5,6b}$ = 4.9 Hz, H-6b), 4.13 (td, 1 H, $J_{4,5}$ = 9.0 Hz, H-5), 3.92 (ddd, 1 H, $J_{3,F}$ = 30.9 Hz, $J_{3,4}$ = 9.8 Hz, H-3), 3.68 (t, 1 H, H-4), 3.30–3.16 (m, 2 H, SO_2CH_2), 1.95–1.73 (m, 2 H, $SO_2CH_2CH_2$), 1.51–1.22 (m, 18 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 158.9 (CO), 88.1 (C-2, d, $J_{C2,F}$ = 184.8 Hz), 72.5 (C-3, d, $J_{C3,F}$ = 18.9 Hz), 71.7 (C-1, d, $J_{C1,F}$ = 23.9 Hz), 70.6 (C-4, d, $J_{C4,F}$ = 3.8 Hz), 68.6 (C-6), 56.9 (C-5), 52.4 (SO_2CH_2), 33.1–22.5 (CH_2), 14.5 (CH_3). ^{19}F NMR (470 MHz, CD_3OD) δ −203.9. ESIMS: *m/z* 468.33 $[M + HCO_2]^-$. Anal. Calcd for $C_{19}H_{34}FNO_6S$: C, 53.88; H, 8.09; N, 3.31; S, 7.57. Found: C, 53.54; H, 7.86; N, 3.02; S, 7.21.

(1*R*)-3-*O*-Benzyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonylnojirimycin (13). Compound **13** was obtained from **56** following the general procedure described above. Purification by column chromatography (1:2 → 1:1 EtOAc-cyclohexane). Yield: 19 mg (73%). R_f 0.39 (2:3 EtOAc-cyclohexane). $[\alpha]_D$ +2.8 (*c* 1.2 in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40–7.27 (m, 5 H, Ph), 5.24 (d, 1 H, $J_{1,2}$ = 6.8 Hz, H-1), 4.96 (d, 1 H, $^2J_{H,H}$ = 11.5 Hz, OCH_2Ph), 4.91 (ddd, 1 H, $J_{2,F}$ = 47.8 Hz, $J_{2,3}$ = 9.2 Hz, H-2), 4.66 (d, 1 H, OCH_2Ph), 4.56 (t, 1 H, $J_{6a,6b}$ = $J_{5,6a}$ = 9.0 Hz, H-6a), 4.42 (bq, 1 H, $J_{3,4}$ = $J_{3,F}$ = 9.2 Hz, H-3), 4.25 (dd, 1 H, $J_{5,6b}$ = 5.3 Hz, H-6b), 4.14 (ddd, 1 H, $J_{4,5}$ = 9.5 Hz, H-5), 3.42 (t, 1 H, H-4), 3.14 (t, 2 H, *J* = 8.0 Hz, SO_2CH_2), 1.97–1.80 (m, 2 H, $SO_2CH_2CH_2$), 1.47–1.21 (m, 18 H, CH_2), 0.87 (t, 3 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 155.9 (CO), 137.5–128.2 (Ph), 90.1 (C-2, d, $J_{C2,F}$ = 192.1 Hz), 79.1 (C-3, d, $J_{C3,F}$ = 16.5 Hz), 75.5 (OCH_2Ph, d, *J* = 3.0 Hz), 72.8 (C-4, d, $J_{C4,F}$ = 7.8 Hz), 67.9 (C-1, d, $J_{C1,F}$ = 26.6 Hz), 66.9 (C-6), 54.6 (*J* = 4.8 Hz, SO_2CH_2), 53.8 (C-5), 31.9–21.4 (CH_2), 14.1 (CH_3). ^{19}F NMR (470 MHz, $CDCl_3$) δ −197.9. ESIMS: *m/z* 558.37 $[M + HCO_2]^-$. Anal. Calcd for $C_{26}H_{40}FNO_6S$: C, 60.80; H, 7.85; F, 3.70; N, 2.73; S, 6.24. Found: C, 60.59; H, 7.56; N, 2.48; S, 5.95.

(1*R*)-3-*O*-Benzyl-2-deoxy-1-*S*-dodecyl-2-fluoro-5*N*,6*O*-oxomethylidene-1-sulfonylmannonojirimycin (17). Compound **17** was obtained from **57** following the general procedure described above. Purification by column chromatography (1:3 → 1:2 EtOAc-cyclohexane). Yield: 25 mg (97%). R_f 0.35 (1:2 EtOAc-cyclohexane). $[\alpha]_D$ −14.9 (*c* 1.0 in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45–7.30 (m, 5 H, Ph), 5.56 (dt, 1 H, $J_{2,F}$ = 47.6 Hz, $J_{2,3}$ = $J_{1,2}$ = 1.6 Hz, H-2), 5.27 (dd, 1 H, $J_{1,F}$ = 16.4 Hz, H-1), 4.81 (d, 1 H, $^2J_{H,H}$ = 11.4 Hz, OCH_2Ph), 4.63 (d, 1 H, OCH_2Ph), 4.60 (t, 1 H, $J_{6a,6b}$ = $J_{5,6a}$ = 9.0 Hz, H-6a), 4.38 (dd, 1 H, $J_{5,6b}$ = 5.1 Hz, H-6b), 4.21 (td, 1 H, $J_{4,5}$ = 8.7 Hz, H-5), 3.92–3.82 (m, 2 H, H-3, H-4), 3.12 (ddd, 1 H, $^2J_{H,H}$ = 14.1 Hz, $^3J_{H,H}$ = 10.4 Hz, $^3J_{H,H}$ = 5.7 Hz, SO_2CH_2), 3.05 (ddd, 1 H, SO_2CH_2), 1.95–1.77 (m, 2 H, $SO_2CH_2CH_2$), 1.47–1.21 (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 156.7 (CO), 136.6–128.3 (Ph), 82.1 (C-2, d, $J_{C2,F}$ = 189.8 Hz), 78.7 (C-3, d, $J_{C3,F}$ = 18.1 Hz), 72.6 (OCH_2Ph), 70.4 (C-1, d, $J_{C1,F}$ = 23.2 Hz), 68.4 (C-4, d, $J_{C4,F}$ = 3.7 Hz), 67.2 (C-6), 54.7 (C-5), 51.9 (SO_2CH_2), 31.9–21.5 (CH_2), 14.1 (CH_3). ^{19}F NMR (282 MHz, $CDCl_3$) δ −202.4. ESIMS: *m/z* 558.33 $[M + HCO_2]^-$. Anal. Calcd for $C_{26}H_{40}FNO_6S$: C, 60.80; H, 7.85; N, 2.73; S, 6.24. Found: C, 60.65; H, 7.59; N, 2.48; S, 5.97.

4.3. Cell lines and growth conditions

In this study, we used the following human solid tumor cell lines kindly provided by Dr. Godefridus J. Peters (Cancer Center Amsterdam, Vrije Universiteit, Amsterdam, The Netherlands): A549 (non-small cell lung), HBL-100 (breast), and HeLa (cervix), SW1573 (non-small cell lung), and its P-gp overexpressing variant (SW1573/Pgp), T-47D (breast), and WiDr (colon). In addition, the BJ-hTERT human fibroblast cells were given by Dr. Raimundo Freire (Universidad de La Laguna, Canary Islands). The cell culture media was RPMI 1640 supplemented with 1 mM glutamine, 5% FBS and antibiotics. Cells were grown at 37 °C in a humidified atmosphere of 5% CO_2 and maintained at low passage.

4.3.1. Growth inhibition assays

The anti-proliferative activity of compounds was tested using our implementation of the protocol of the National Cancer Institute (NCI) of the USA. The following seeding densities (cells per well) were used:

2500 (A549, HBL-100, HeLa and SW1573), 5000 (T-47D, WiDr and SW1573/PGP), and 7000 (BJ-hTERT). Samples for testing were dissolved initially in DMSO at 40 mM (400 times the maximum test concentration). Verapamil (stock solution 40 mM in DMSO) was used as a P-gp transport inhibitor in SW1573/P-gp experiments. PTX (stock solution 4 mM in DMSO) was used as positive control in SW1573/P-gp experiments. For each test compound, the cells were exposed for a period of 48 h to serial decimal dilutions of the test compounds (0.001–100 μM). Cell culture medium containing verapamil was prepared by adding the final concentration of verapamil of 10 μM. For each product GI_{50} values were calculated according to the NCI formulas.

4.3.2. Colony formation (clonogenic) assay

HeLa and SW1573 cells were seeded (400 cells/well) onto 6 well plates. On the next day, compound **11** was added at a low (GI_{50}/3) and a high (GI_{50}) dose, based on the GI_{50} values for each cell line. Cells were exposed for seven days, then fixed with methanol and stained using crystal violet (0.5% w/v in deionized water). Colonies were counted using AutoCellSeg, a MatLab implementation for automatic segmentation [49].

4.3.3. Label-free continuous live cell imaging

HeLa and SW1573 cells were seeded (100,000 cells/dish) onto 35 mm high glass-bottom μ-dish (IBIDI, Germany). After 24 h, the medium was changed for RPMI 1640 without phenol red, and the cells were treated with compound **11** at 20 μM. Immediately, the plates were placed in the CX-A label-free cell imaging system (Nanolive S.A., Switzerland). The images from a 236 μm × 236 μm area were taken every 3 min for 15 h. After image acquisition, the Eve segmentation and analysis software (Nanolive S.A., Switzerland) was used to evaluate the average dry mass density. The measurements were obtained for each cell at all of the imaging cycles for each treatment. The initial cell amount of the field area was in the range 10–20 cells.

4.4. Procedure for anti-inflammatory assays

4.4.1. Cell culture

Mouse microglia Bv.2 cell line was purchased to (ACCEGEN Biotechnology Fairfield, NJ 07004 USA). An amount of 1.5105 cells/well were seeded in a 6-multiwell plate (Sarstedt, Germany). The culture conditions were 37 °C in a humidified atmosphere with 5% CO_2 in RPMI supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 1% (v/v) penicillin/streptomycin (Sigma), and 2 mM L-glutamine (Gibco, Carlsbad, CA, USA). All experimental cell approaches were performed in complete medium without FBS.

4.4.2. Analysis of the cellular viability by crystal violet staining

Cells were cultured in 24-well plates and grown up to 70% confluence. The cells were treated with **10** , **11** , **13** , **18** and **19** to reach final concentrations of 0.1, 1.0, 10 and 25 μM, and incubated in serum-free medium. After 24 h, the medium was discarded and cells were fixed by adding 0.5 mL of glutaraldehyde 1% (v/v) for 30 min. Then, the plates were rinsed with phosphate buffer saline (PBS) and the remaining viable adherent cells were stained with crystal violet 0.1% (w/v) for 30 min. After rinsing plates with water and drying for 24 h, 0.5 mL of acetic acid 10% (v/v) were added. The absorbance of each plate was read spectrophotometrically at 590 nm in a microplate reader (Versamax Tunable Microplate reader, Molecular Devices, Sunnyvale, CA, USA).

4.4.3. Analysis of nitrites (NO_2^-)

Cells were cultured in 6-well plates and grown up to 70% confluence. The cells were pre-treated for 3 h with **10** , **11** , **13** , **18** and **19** at 10 μM in serum-free medium and then stimulated with LPS (200 ng/mL) for another 24 h. After cell treatments, levels of NO_2^- were measured by using the Griess method [50]. Briefly, cell cultured medium was treated with an acid solution containing 1% sulphanilamide and 0.1% *N*-(1-naphthyl)ethylenediamine (NEDA) and read spectrophotometrically at 548 nm in a microplate reader.

4.4.4. Quantitative real-time PCR (qPCR) analysis

Total RNA was extracted with TRIzol® reagent (Invitrogen, Madrid, Spain) and reverse-transcribed using the iScript gDNA Clear cDNA Synthesis Kit from BioRad (Madrid, Spain). qPCR was performed with the iTaq Universal Probes Supermix from BioRad (Madrid, Spain) in a CFX Connect Real-Time System from BioRad (Madrid, Spain). Analysis of relative gene expression data were performed using the $2^{-\Delta\Delta CT}$ method. Primer probe sets for mouse *Il1b*, *Tnfa*, and *Actinb* were purchased as predesigned TaqMan gene expression assays (Applied Biosystems, Foster City, CA, USA).

4.4.5. Western Blot

Proteins were resolved using denaturing SDS-PAGE and transferred to PVDF membranes (Bio-Rad). Membranes were blocked using 5% nonfat dried milk or 3% BSA in 10 mM Tris-HCl, 150 mM NaCl, pH 7.5 (TBS), and incubated overnight with anti p-P38-MAPK or P38-MAPK (1:1000) in 0.05% Tween-20-TBS. Immunoreactive bands were visualized using the enhanced chemiluminescence reagent (Bio-Rad).

4.4.6. Statistical analysis

Statistical Analysis Western blot quantification was performed using the ImageJ program. Data are presented as mean standard deviation (SD) and were compared by using the Bonferroni ANOVA test. All statistical analyses were performed using GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA) with 2-sided tests. Differences were considered statistically significant at $p \leq 0.05$.

4.5. Molecular docking experiments

The protein structure of the p38α MAPK receptor was obtained from Protein Data Bank in complex with PIA23 (PDB ID 4E6A). The protein-ligand docking calculations were performed with the AutoDock Vina software [51]. The protein was prepared following the Protein Preparation Wizard of Schrödinger Software in Maestro [52]. Hydrogen atoms were added under physiological conditions (pH 7). The initial coordinates of ligands were built with Maestro program, assuming a $^4C_1$ conformation for the six-membered ring. A previous energy minimization using OPLS4 force field was used to optimize the ligand structures. For the docking calculations, the volume for exploration was defined by a grid box centered at a point in the middle of the hydrophobic cavity (x = 6.5, y = 2.0, z = −2.2, according to PDB ID 4E6A coordinates), with 58 × 67 x 75 points (22 × 26 × 28 Å) and a grid spacing of 0.375 Å. Kollman charges were added to the protein using AutodockTools 1.5.6 software. The Lamarckian genetic algorithm was used with rigid side chains for the receptor and randomly changing the torsion angles (16 rotable bonds for compound **13**) and overall orientation of the ligand. Autodock Vina generated docking results for the top 9 poses in the pdbqt format for each ligand and the data were analyzed in Pymol software. For comparison, docking calculations of PIA23 that reproduced both the binding conformation and hydrogen bonds of PIA23 in a cocrystallized structure with p38α MAPK (PDB ID 4E6A) was carried out. Molecular representations in figures were generated with the Pymol software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Supplementary Material

Acknowledgments

This work was supported by grants from the Ministerio de Ciencia e Innovación, the Agencia Estatal de Investigación and the European Regional Development Funds (PID2019-105858RB-I00) to COM. MCP-P is a FPU fellow (Grant number FPU19/04361). EMS-F thanks the VII Plan Propio de Investigación y Transferencia from the University of Seville (VIIPPIT-2022-V.1, Modality A2). JMGF was supported by grants from the Ministerio de Ciencia, Innovación y Universidades, the Agencia Estatal de Investigación and the European Regional Development Funds (RTI2018-097609-B-C21 and PID2021-124247OB-C21) and from the Junta de Andalucía and the European Regional Development Funds (P20_00166). Technical assistance from the research support services of the University of Seville (CITIUS) is also acknowledged. AG-B, AP and JMP thank the Spanish Government (Project PID2021-123059OB-I00 funded by MCIN/AEI/10.13039/501100011033/FEDER, UE). AP thanks the EU Social Fund (FSE) and the Canary Islands ACIISI for a predoctoral grant TESIS2020010055. AIA was supported by grants from Instituto de Salud Carlos III (PI22/01718), Consejería de Universidad, Investigación e Innovación de la Junta de Andalucía (PI20-01331) and Convocatoria de Subvenciones para la Financiación de la Investigación y la Innovación Biomédica y en Ciencias de la Salud en el Marco de la Iniciativa Territorial Integrada 2014-2020 para la Provincia de Cádiz, Fondos ITIFEDER. FM-L is FPI fellow (Grant number PI20-0036).

Supporting information available

Cytotoxic effect of different concentrations of DX on the Bv.2 cell line viability (Fig. S1). Anti-inflammatory effect of selected sp^2-iminosugars (**10** , **11** , **13** , **18** and **19**) at different doses in LPS-stimulated Bv.2 cells (Fig. S2). NMR spectra for all new compounds (Figs. S3–S44). Video S1: Control (untreated) HeLa cells; Video S2: **11** -treated HeLa cells; Video S3: Control (untreated) SW1573 cells; Video S4: **11** -treated SW1573 cells.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2023.115390.

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