Matrix Metalloproteinase-12 Inhibitors: Synthesis, Structure-Activity Relationships and Intestinal Absorption of novel Sugar-Based Biphenylsulfonamide Carboxylates

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Abstract

MMP-12 is a validated target in pulmonary and cardiovascular diseases. The principal obstacles to clinical development of MMP-12 inhibitors are an inadequate selectivity for the target enzyme and a poor water solubility, with consequent poor oral bioavailability. We recently reported a new class of sugar-based arylsulfonamide carboxylates with a nanomolar activity for MMP-12, a good selectivity and an improved water solubility. In this study, we designed and synthesized new derivatives to characterize the structure-activity relationship (SAR) within this class of glycoconjugate inhibitors. All the new derivatives were tested on human recombinant MMP-12 and MMP-9 in order to evaluate their affinity and the selectivity for the target enzyme. Among them, the four most promising compounds were selected to assess their intestinal permeability using an ex vivo everted gut sac model. Given the

high polarity and structural similarity to glucose, compound **3** was demonstrated to cross the intestinal membrane by using the facilitative GLUT2 transport.

1. Introduction

MMP-12, or macrophage metalloelastase, belongs to the matrix metalloproteinases (MMPs), a family of endopeptidases able to degrade the structural components of the extracellular matrix (ECM).[1] MMP-12 can cleave a broad spectrum of extracellular matrix components including elastin, the major constituent of alveolar walls, and other proteins such as type IV collagen, fibronectin, laminin, gelatin, vitronectin, and chondroitin sulfates.[2] This enzyme is mainly produced by macrophages and is involved in acute and chronic pulmonary inflammatory diseases associated with intense airway remodeling, such as chronic obstructive pulmonary disease (COPD) and emphysema.[3] Moreover, MMP-12 has been shown to be critical in the initiation and progression of atherosclerosis by stimulating the transformation of fatty streaks into fibrous plaques.[4] Given its upregulation in atherosclerosis,[5] aneurysms,[6] and cardiovascular diseases,[7] MMP-12 selective tracers for molecular imaging have been recently developed.[8] Similarly, several MMP-12 selective inhibitors have been reported and are being tested in various preclinical trials for the treatment of lung[9] and cardiovascular[10] diseases. The principal obstacles to clinical development of MMP inhibitors (MMPIs) are an inadequate selectivity for the target enzyme and a poor water solubility, with consequent poor oral bioavailability.

To overcome these problems, in the last years some carbohydrate-containing MMPIs were developed in order to obtain more bioavailable derivatives containing the classical saccharide moiety but also the more specific azasugars.[11,12,13] Unfortunately, these novel MMPIs were characterized by an increased hydrophilicity but a low selectivity for a specific MMP.

In a previous work,[14] we reported the synthesis and the biological activity of new sugar-based arylsulfonamide carboxylates as MMP-12 inhibitors. These glycoconjugates were prepared starting from the previously described carboxylate MMP-12 inhibitor **1**[15] (Figure 1) through the insertion of a β-*N*-acetyl-D-glucosamine (GlcNAc) moiety in P2'. In particular, compounds **2** and **3** (Figure 1) were able to conjugate a nanomolar activity for MMP-12, with a good selectivity and an improved water solubility with respect to derivative **1**, devoid of the sugar moiety.

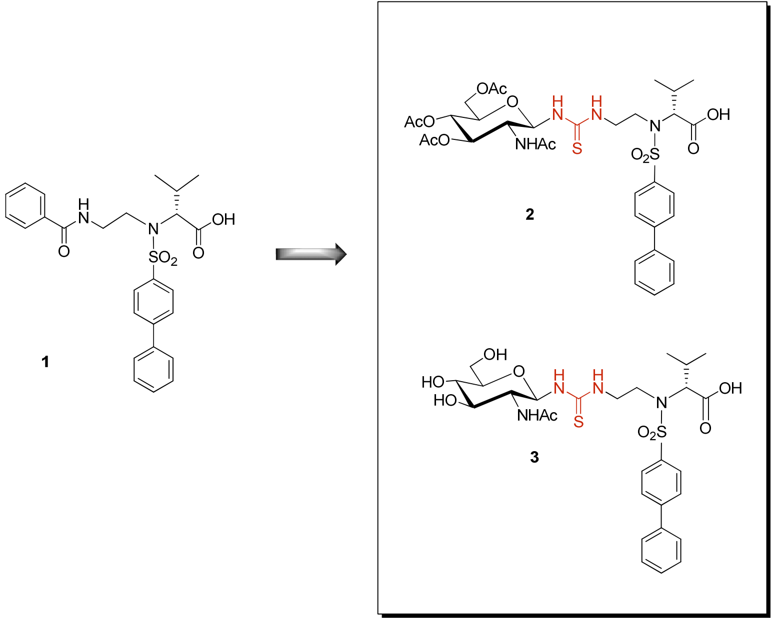

Figure 1. Chemical structure of selective MMP-12 inhibitors **1-3**.

On the basis of these encouraging results, in the present study we undertook the synthesis of new glycoconjugate inhibitors of MMP-12 (**4-15**, Figure 2) in order to perform a Structure-Activity Relationship (SAR) analysis for this class of compounds. In fact, a SAR analysis could be useful to further optimize the glycoconjugate inhibitors already developed. The modifications included the introduction of a different glycosidic moiety, by replacing *N*-acetyl glucosamine with glucose, the change of the conjugation position (from C-1 to C-6 sugar position) and the spacer length. In particular, the conjugation of the sugar to the MMP inhibitor scaffold was obtained through two different strategies: by Mitsunobu reaction and by thioureidic linker formation (Figure 2).

Figure 2. General structure of new glycoconjugates **4-15**.

All the new derivatives were tested by fluorometric assay on human recombinant MMP-12 and MMP-9 in order to evaluate their affinity and the selectivity for the target enzyme. Among them, the most promising compounds were selected to assess their intestinal permeability using an *ex vivo* everted gut sac model. In fact, it is well known that intestinal absorption of a drug after oral administration relies on both solubility and the ability to pass gastrointestinal (GI) membranes. At this regard, intestinal permeability is referred to the ability of a compound to penetrate through the intestinal epithelium; then it is a critical parameter of absorption. The quantification of permeated compounds has been performed by high performance liquid chromatography (HPLC) with ultraviolet (UV) detection, a commonly used technique that has been successfully applied to the determination of the intestinal permeation of drugs.[16] Finally, we investigated the absorption mechanism of glycoconjugate inhibitors of MMP-12 in the *ex vivo* model. On the basis of the well-known glucose transporter mechanisms present at the intestinal level, inhibition studies were conducted using the pharmacological agents phloridzin, a potent inhibitor of sodium-dependent glucose transporter 1 (SGLT1), or phloretin, an inhibitor of glucose transporter 2 (GLUT2).[17]

2. Results and Discussion

2.1. Design of new glycoconjugate MMP-12 inhibitors

In order to develop a SAR study for this class of MMP-12 inhibitors, the previously described thioureido derivatives **2** and **3** were selected as starting point. In particular, compound **3** showed a nanomolar activity on MMP-12 (IC_{50}= 40 nM) and a good selectivity over MMPs with improved hydrophilicity (water solubility > 5 mM and clog *P* = 3.15).[14] This hit compound presents a carboxylate as zinc-binding group (ZBG) able to chelate the catalytic zinc ion and a biphenyl sulfonamide scaffold able to interact with some fundamental aminoacid residues of the MMP-12 active site (PDB ID: 5I3M). Moreover, in this compound a β-*N*-acetyl-D-glucosamine residue is linked to the sulfonamide nitrogen atom (P2' substituent) through insertion of a thioureido group that is biocompatible and stable in most biological systems.

In the present work, a substitution of *N*-acetyl glucosamine with glucose was undertaken in order to promote intestinal absorption of the new compounds by exploiting the glucose transporters. Since glucose is actively absorbed by sodium-dependent glucose cotransporter 1 (SGLT1) in the brush-border membrane (BBM) of intestinal epithelial cells,[18] SGLT1 could also be involved in the transport of some sugar analogues. Sugar-protected analogues, acetylated on the hydroxy groups, were also synthesized and tested on isolated enzymes. Moreover, the influence of the conjugation position on activity was evaluated by linking the MMP inhibitor scaffold in C-6 or in C-1 position on the sugar moiety with or without insertion of the thioureido group.

2.2. Chemistry

The new glycoconjugates **4-15** were synthesized as reported in Scheme 1, 2 and 3. These new compounds were prepared following two different conjugation strategies: by Mitzsunobu reaction (Scheme 1) or by the insertion of a thioureidic linker (Scheme 2 and 3).

The synthesis of glycoconjugates **19**, **20**, **23** and **24** is described in Scheme 1. A Mitsunobu condensation of sulfonamide **18**[14] with alcohols **16**,[19] **17**,[19] **21**[20] and **22**[21] in the presence of diisopropyl azodicarboxylate (DIAD) and triphenylphosphine (PPh_3) gave glycoconjugates **19**, **20**, **23** and **24** (42-55% yields).

18

16: D-glucosamine: R=NHAc
17: D-gluco: R=OAc

19: R=NHAc;
20: R=OAc;

4: R=NHAc;
5: R=OAc;

6: R=NHAc
7: R=OH

21: R=NHAc
22: R=OAc

23: R=NHAc
24: R=OAc

8: R=NHAc
9: R=OAc

10: R=NHAc
11: R=OH

Scheme 1. Reagents and conditions: i) **18**, PPh_3, DIAD or DMEAD, THF dry, rt, 42-55%; ii) TFA, DCM dry, rt, 89-92%; iii) NH_3-MeOH 7N, MeOH, rt, 85-90%.

25

26

27

28

29

12: R= Ac
13: R=H

Scheme 2. Reagents and conditions: i) NaN_3, DMF, rt (quantitative); ii) PPh_3.resin, THF-H_2O 20:1, rt; iii) DPT, DCM, rt (22% for two steps); iv) **18**, Et_3N, DCM, 73%; v) TFA, DCM, 42%; vi) NH_3-MeOH 7N, MeOH, 75%.

The β-azido glucose derivative **25** was obtained by conversion of the commercial 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide through S_N2 reaction (quantitative yield). The β-glucose isothiocyanate **27** (Scheme 2) was prepared starting from β-azido glucose **25**. The azido derivative **25**[22] was reduced to the corresponding amine **26** by Staudinger reaction using PPh_3 in THF dry and without further purification was transformed into the corresponding isothiocyanate derivative **27** by treatment with di-2-pyridyl thionocarbonate (DPT) (22% yield from **25**).

Scheme 3. Reagents and conditions: i) DPT, DCM, rt, 78%; ii) H_2/Pd, MeOH/DCM/H_2O, rt, 50%; iii) **32**, Et_3N, DCM, 47%; iv) TFA, DCM, 52.5%; v) NH_3-MeOH 7N, MeOH,73%.

The C-6 azido D-glucose **31** (Scheme 3) was prepared following the reported procedure.[23] The azido functionality of **31** was reduced to the corresponding amine **32** by Pd catalyzed hydrogenation (50% yield). Thioureido derivatives **29** and **33** (Scheme 2 and 3) were obtained through direct coupling of trifluoroacetate salt MMP inhibitor amino **28**[14] with the isothiocyanate β-glucose derivative **27** (Scheme 2) or by conjugation of the C-6 amino glucose **32** with the isothiocyanate MMP inhibitor **30** (78% yield, Scheme 3), obtained by treatment of **28** with DPT as described above for the

isothiocyanate **27**. The condensations between isothiocyanates **27** or **30** and amines **28** or **32** were performed in DCM and in presence of triethylamine (Et_3N) at 60 °C (47-73% yields). Purification of the crude products afforded pure esters **29** and **33**.

Removal of the *tert*-butyl group of esters **19, 20, 23, 24, 29** and **33** by treatment with trifluoroacetic acid (TFA) gave the desired carboxylic acids **4**, **5, 8**, **9**, **12** and **14** after purification by flash chromatography. Finally, the desacetylation of **4**, **5, 8**, **9**, **12** and **14** obtained by basic hydrolysis with 3.5 N NH_3-MeOH afforded deprotected derivatives **6**, **7**, **10**, **11**, **13** and **15** in good yields.

2.3. MMP inhibition studies

The series of new glycoconjugate arylsulfonamide carboxylates, the sulfonamides **4-11** (Table 1) devoid of the ethylene thioureido spacer and the thioureido derivatives **12-15** and (Table 2), were screened *in vitro* on human recombinant MMP-12 and MMP-9 by a fluorometric assay. An improved selectivity for MMP-12 over MMP-9 could be indicative of the ability of the new derivatives to discriminate among MMPs given the high homology between the catalytic domains of these two enzymes both characterized by a "medium" S1' pocket.[24] The glycoconjugates **2** and **3** were used as reference compounds.

Analyzing the sulfonamide series, those compounds functionalized on the anomeric position (**4-7**) showed a decreased activity on MMP-12 (IC_{50} = 1.3 - 40 μM) with respect to the reference compounds **2** and **3** (IC_{50} = 18 - 40 nM). These results indicate that the insertion of a spacer in the anomeric position is fundamental to have a nanomolar affinity for the target enzyme.

In the same series, it is noteworthy the difference of MMP-12 affinity due to the nature of the sugar moiety, in fact the glucose glycoconjugates **5** and **7** presented a better activity than the corresponding *N*-acetyl glucosamine derivatives **4** and **6**, respectively. In particular, the protected glucose carboxylate **5** was 4-fold more active than the glucosamine derivative **4** and the deprotected glucose derivative **7** was 7-fold more active than **6**. Finally, the modification of the conjugation position was fundamental, considering the significant improvement of MMP-12 activity for C-6 derivatives. In fact, the C-6 glycoconjugates **9** and **11** presented a nanomolar activity for MMP-12 with a 114-fold improvement of activity for carboxylic acid **9** with respect to its C-1 substituted analogue **5** and with a 25-fold improved activity of the deprotected **11** compared to its C-1 substituted analogue **7**. These results confirmed also the need for a flexible linker between the MMP inhibitor scaffold and the sugar moiety in the sulfonamide series. This condition is satisfied for compounds **8-11** showing a direct conjugation in C-6 position but not for compounds **4-7** with a direct conjugation in the anomeric position. The best

compound of this series was the C-6 GlcNAc derivative **10**, with an activity on MMP-12 comparable to **3** (IC_{50} = 41 nM) but a lower selectivity over MMP-9.

Table 1. MMP-12 and MMP-9 inhibitory activity (IC_{50} nM values) of sulfonamido derivatives **4-11.**

		IC_{50} (nM)[a]	
Compd	**R**	**MMP-12**	**MMP-9**
4	OAc, AcO, AcO, O, NHAc	40000	nd
5	OAc, AcO, AcO, O, OAc	9600	nd
6	OH, HO, HO, O, NHAc	9200	nd
7	OH, HO, HO, O, OH	1300	>50000
8	AcO, AcO, O, OMe, NHAc	83	1235
9	AcO, AcO, O, OMe, OAc	84	2600
10	HO, HO, O, OMe, NHAc	41	890
11	HO, HO, O, OMe, OH	51	2200
2		18	1200
3		40	5400

[a] IC_{50}values are the average of three determinations with a standard deviation of <10%; nd: not determined

In the thioureido series (Table 2) the C-1 glycoconjugates **12** and **13**, glucose analogues of the reference compounds **2** and **3**, showed a nanomolar activity for MMP-12. In particular, their MMP-12 affinity was comparable to the one of the reference compounds. Moreover, the higher activity of the

acetylated derivative **12** with respect to the deprotected **13** confirmed the data already obtained for compounds **2** and **3**. These results indicated that in the thioureido series of compounds the nature of the sugar moiety does not affect the affinity for MMP-12 (see **12** and **13** compared to **2** and **3**). Moreover, in this series of compounds all presenting a flexible ethylene thioureido linker, the conjugation position does not have a strong impact on MMP-12 inhibitory activity (see **14** and **15** compared to **12** and **13**). However, the conjugation position was crucial for determining the selectivity of thioureidic derivatives. In fact, the C-6 glucoconjugates **14** and **15** revealed a similar inhibitory activity with respect to **12** and **13** but a significantly decreased selectivity for MMP-12 over MMP-9. This decrease of selectivity, namely due to an improved activity on MMP-9, could be explained on the basis of the crystal structure previously obtained for compound **2** co-crystallized with the catalytic domain of MMP-12 and MMP-9.[14] The MMP-9 catalytic site did not allow a proper positioning of the sugar moiety of **2** but the same moiety present in **14** conjugated in C-6 position instead of in C-1 could restore a good binding with the molecular surface of MMP-9.

Table 2. MMP-12 and MMP-9 inhibitory activity (IC_{50} nM values) of thioureido derivatives **12-15** and the reference compounds **2-3.**

		IC_{50} (nM)[a]	
Compd	**R**	**MMP-12**	**MMP-9**
12		19	880
13		98	1120
14		20	32
15		65	520
2		18	1200
3		40	5400

[a]IC_{50} Values are the average of three determinations with a standard deviation of <10%.

Overall, this SAR analysis allowed us to conclude that the most promising compounds obtained so far were the C-1 thioureido derivatives **2** and **3** and their glucose analogues **12** and **13**. Given their activity and selectivity for MMP-12, these four compounds were selected for further studies to evaluate their intestinal permeability.

The physicochemical properties, i.e. octanol/water partition coefficient (cLogP) and topological polar surface area (TPSA), of the most promising new compounds **12** and **13** were predicted *in silico* in comparison with **2** and **3** by using OSIRIS Property Explorer (Table 3). The deprotected derivatives **3**

and **13** presented a particularly low cLogP value, probably due to the presence of the desacetylated glycosidic residues. Futhermore, the permeability properties evaluated by the TPSA parameter, presented quite high values for all compounds. Molecular polar surface area (PSA), is a descriptor that was shown to correlate well with passive molecular transport through membranes and, therefore, it is used to predict the transport properties of drugs.[25] These results agree with our hypothesis that the highly polar glycosylated derivatives could cross the GI membrane not by passive diffusion but through an active transport mechanism.

Table 3. Predicted physicochemical properties of compounds **2**, **3**, **12** and **13**.

Compd	cLogP[*a*]	TPSA[*a*]
12	1.99	253.4
13	0.05	238.2
2	1.51	256.4
3	0.06	229.3

[*a*] clogP (o/w) and TPSA values were calculated by using OSIRIS Property Explorer[26]

2.4. Permeability studies

Everted gut sac model is a efficient tool for studying *ex vivo* drug absorption mechanisms, intestinal metabolism of drugs, role of transporter in drug absorption, and for investigating the role of intestinal enzymes during drug transport through the intestine;[27] therefore we here used it to evaluate the intestinal permeability of the selected compounds. In these experimental conditions (see Experimental section for details), the non-glycoconjugated compound **1**, as well as the glycoconjugated **2**, **12** and **13**, were poorly absorbed through intestinal membrane. Indeed, the maximal concentration detected for these compounds used at 100 µM was lower than 10 µM after 60 min of incubation (Figure 3 and Table 4).

On the other hand, compound **3** (100 µM) showed a better intestinal permeability. Indeed, after 60 min of incubation its concentration inside the sacs was 19.51 ± 2.27 µM. Since compound **3** demonstrated to be the most promising among new MMP-12 inhibitors, a further evaluation of its transport mechanism was carried out.

Glucose and glucose analogues cross the cell membranes with the help of two transporters belonging either to the family of facilitative glucose transporters (GLUTs) or to the family of sodium glucose cotransporters (SGLUTs). In particular, at the intestinal level SGLUT1 is located in brush border

membranes, whereas in basolateral membranes are located GLUT2 transporters. A second series of experiments showed that SGLUT1 is not involved in the transport of compound **3**, since the addition of phloridzin 10 μM as well as 100 μM did not inhibit the adsorption of **3** through intestinal membrane. Instead, the addition of phloretin 100 μM, a known GLUT2 inhibitor, significantly reduced the passage of compound **3** suggesting that it uses the facilitative GLUT2 transport to cross intestinal membrane (Figure 4 and Table 5).

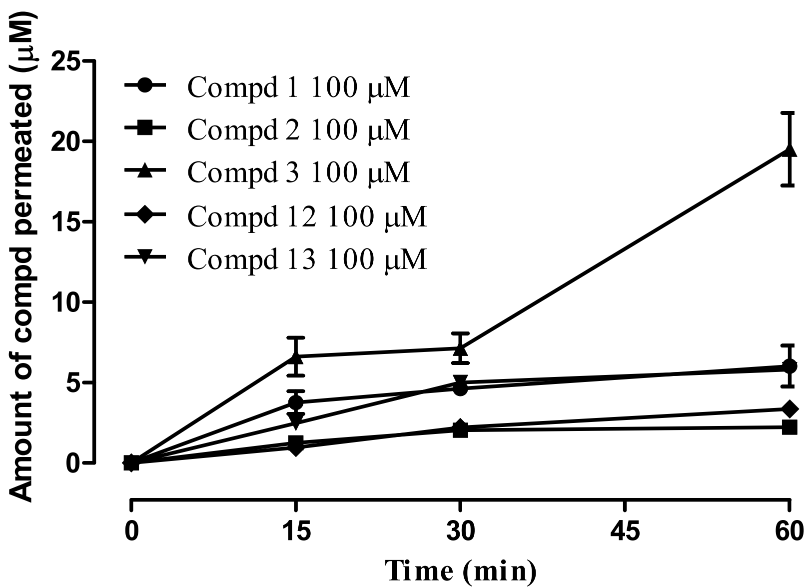

Figure 3. Intestinal permeability of selected MMP-12 inhibitors in Everted Gut Sac model.
Data shown are 6 ± 2.

Table 4. Amounts of compound permeated through intestinal membrane (μM). Data are shown ± SEM.

	Compd 1	Compd 2	Compd 3	Compd 12	Compd 13
15 min	3.87 ± 0.71	1.25 ± 0.07	6.62 ± 1.18	0.97 ± 0.16	2.48 ± 0.04
30 min	4.63 ± 0.24	2.04 ± 0.30	7.14 ± 0.91	2.21 ± 0.17	5.0 ± 0.43
60 min	6.03 ± 1.27	2.23 ± 0.16	19.51 ± 2.27	3.37 ± 0.32	5.82 ± 0.26

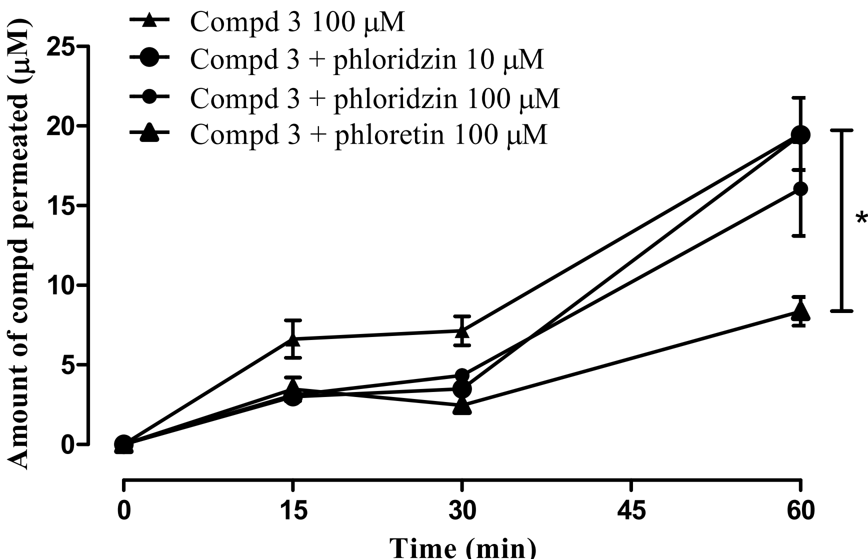

Figure 4. Intestinal permeability of compound **3** in the presence of phloridzin (10 µM and 100 µM), phloretin (100 µM), or in their absence. Data shown are 8 ± 3 and they were analyzed with two-way ANOVA test. The asterisk indicates a statistically significant difference vs pre-incubation with inhibitor (*p<0.05).

Table 5. Amounts of compound permeated through intestinal membrane (µM). Data are shown ± SEM.

	Compd 3	**Compd 3 + phloridizin 10** µM	**Compd 3 + phloridzin 100** µM	**Compd 3 + phloretin 100** µM
15 min	3.87 ± 0.71	3.0 ± 0.06	3.1 ± 0.15	3.47 ± 0.74
30 min	4.63 ± 0.24	3.48 ± 0.41	4.33 ± 0.09	2.47 ± 0.12
60 min	6.03 ± 1.27	19.45 ± 0.15	16.05 ± 2.95	8.37 ± 0.9

To quantify the intestinal permeability of the selected compounds **2**, **3**, **12** and **13**, in comparison with reference compound **1**, analytical HPLC methods have been developed. For each compound, a calibration curve at different concentration in the range of 500-1 µM has been prepared. Good linearity between the peak-area and the respective concentration of compounds was observed, and the correlation coefficients (r^2) for the selected compounds were in the range $0.984 \leq r^2 \leq 0.999$.

The selectivity of the method has been determined by testing the presence in the medium of other substances that could interfere with the peak of the compound in the chromatogram. Therefore, chromatograms were compared, and in the chromatographic conditions used for the analysis no other interfering peaks were observed. The lower limit of quantification was 0.7 μM. The methods have been demonstrated to be sensitive and accurate for the determination of the compounds **1**, **2**, **3**, **12** and **13**.

The developed methods have been used to analyze samples derived from *ex vivo* everted gut sac permeation experiments. The samples of serosal fluid at the selected time point (0, 15, 30, 60 min) were injected in the HPLC system and the concentration of the compounds has been directly determined from the linear regression equation derived from the calibration curve.

3. Conclusions

Oral administration of selective MMP-12 inhibitors might be useful to treat pulmonary and cardiovascular diseases. To achieve this purpose, compounds selective for the target enzyme with adequate hydrophilicity should be developed. In this study, we designed and synthesized new glycoconjugates belonging to the class of sugar-based arylsulfonamide carboxylates previously reported as MMP-12 inhibitors. In this class, compounds **2** and **3** were able to conjugate a nanomolar activity for MMP-12, with a good selectivity and an improved water solubility with respect to the parent compound **1**, devoid of the sugar moiety. All new derivatives were tested *in vitro* on human recombinant MMP-12 and MMP-9 by fluorometric assay. The SAR results confirmed that the most promising compounds obtained so far were the C-1 thioureido *N*-acetylglucosamino-based derivatives **2** and **3** and their glucose analogues **12** and **13**. Given their activity and selectivity for MMP-12, these four compounds were selected to assess their intestinal permeability using an ex vivo everted gut sac model as a first step to evaluate their intestinal absorption. In fact, it is well known that intestinal absorption of a drug after oral administration relies on both solubility and the ability to pass gastrointestinal membranes. Among the four glycoconjugates, compound **3** showed the highest intestinal permeability and further experiments were conducted to determine the absorption mechanism. Considering its polarity and structural similarity to glucose an active transport was hypothesized and our results showed that compound **3** crossed the intestinal membrane by using the facilitative GLUT2 transport. Further studies to determine the pharmacokinetic characteristics of this lead compound before *in vivo* testing are ongoing.

4. Experimental Section

4.1. Chemistry. Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 20±2 °C. ^{1}H NMR spectra were recorded in appropriate solvents with a Bruker Avance II operating at 250.12 MHz or a Bruker Avance III HD 400 spectrometer operating at 400 MHz. ^{13}C NMR spectra were recorded with the spectrometers operating at 62.9 MHz or 100.57 MHz. The assignments were made, when possible, with the aid of DEPT, COSY, HSQC experiments. The first order proton chemical shifts are referenced to residual solvents and *J*-values are given in Hz. All reactions were followed by TLC on Kieselgel 60 F_{254} with detection by UV light and/or with ethanolic 10% phosphomolybdic or sulfuric acid, and heating. Kieselgel 60 (Merck, 70-230 and 230-400 mesh, respectively) was used for column and flash chromatography. Some of flash chromatography were conducted by the automated system Isolera Four SV™ (Biotage®), equipped with UV detector with variable wavelength (200-400 nm) or using prepacked ISOLUTE Flash Si II cartridges (Biotage). Microwave-assisted reactions were run in a CEM Discover LabMate microwave synthesizer. Unless otherwise noted, solvents and reagents were obtained from commercial suppliers and used without further purification. All reactions involving air- or moisture-sensitive reagents were performed under an argon atmosphere using anhydrous solvents. Anhydrous dimethylformamide (DMF), dichloromethane (CH_2Cl_2), 1,2-dichloethane (DCE) and THF were purchased from Sigma-Aldrich. $MgSO_4$ or Na_2SO_4 were used as the drying agents for solutions. Elemental analysis was used to determine the purity of target compounds. Analytical results are within ± 0.40% of the theoretical values.

4.1.1. General procedure for the synthesis of *tert*-butyl derivatives 19, 20, 23 and 24

To a solution of the appropriate sugar (**16**, **17**, **21** or **22**, 1 equiv) in dry THF, sulfonamide **18** (1.3 equiv) was added under Argon atmosphere. The mixture was cooled down to 0 °C, then dry PPh_3 (3.5 equiv) and di-2-methoxyethylazodicarboxylate (DMEAD) or di-isopropylazodicarboxylate (DIAD) (3.5 equiv) were added. The reaction mixture was stirred at room temperature until TLC analysis revealed a complete disappearance of the saccharide starting material. Subsequently, the solvent was evaporated and the residue was taken up with CH_2Cl_2 and washed with saturated solution of $NaHCO_3$ (1 × 20 mL). The aqueous phase was extracted with CH_2Cl_2 (2× 20 mL). The combined organic phases were dried with Na_2SO_4, filtered and evaporated under reduced pressure. The crude was purified by flash chromatography.

4.1.1.1. *tert*-Butyl (2*R*)-2-(*N*-((2*R*,3*R*,4*S*,5*S*,6*R*)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)biphenyl-4-ylsulfonamido)-3-methylbutanoate (19).

The Mitsunobu reaction was conducted following the general procedure, starting from a solution of compound **16** (40.0 mg, 0.116 mmol) in dry THF (2.36 mL), the biphenylsulfonamide **18** (58.6 mg, 0.150 mmol), PPh_3 (106.0 mg), and DMEAD (94.9 mg, 0.405 mmol). The reaction mixture was stirred at room temperature for 22 h. After work-up, the crude (284 mg) was purified by flash chromatography (*n*-hexane-EtOAc 4:6 + 0.01% Et_3N) affording derivative **19** (39.5 mg, 47% yield) as a clear syrup. ^{1}H NMR (250.12 MHz, CD_3CN): δ 7.97 (m, 2H, 2×Ar-H), 7.79 (m, 2H, 2×Ar-H), 7.67 (m, 2H, 2×Ar-H), 7.54-7.45 (m, 3H, 3×Ar-H), 6.59 (d, 1H, $J_{2,NH}$=8.2 Hz, N*H*Ac), 5.15 (m, 1H, H-3), 5.12 (d, 1H, $J_{1,2}$=9.3 Hz, H-1), 5.04 (m, 1H, H-2), 4.99 (m, 1H, H-4), 4.10 (m, 1H, H-6a), 3.97 (m, 1H, H-6b), 3.88 (d, 1H, *J*=10.2 Hz, *CH*NSO_2), 3.83 (m, 1H, H-5), 2.20 (m, 1H, Me_2*CH*), 1.99, 1.97, 1.94 (3s, each 3H, 3×*Me*COO), 1.72 (s, 3H, *Me*CON), 1.31 (s, 9H, C*Me₃*), 0.92 (d, 3H, J_{vic}=6.5 Hz, CH*Me*₂), 0.79 (d, 3H, J_{vic}=6.5 Hz, CH*Me*₂). ^{13}C NMR (62.9 MHz, CD_3CN): δ 171.2-170.4 (4× C=O), 146.5 (Ar-*C*-SO_2), 139.8, 138.7 (2×Ar-*C*), 130.1-128.2 (Ar-*C*H), 85.7 (C-1), 83.8 (*C*Me_3), 76.3 (C-3), 74.6 (C-5), 69.3 (C-4), 67.6 (*CH*NSO_2), 63.3 (C-6), 53.1 (C-2), 30.1 (*CH*Me_2), 28.0 (C*Me₃*), 23.5 (*Me*CON), 20.9 (3×*Me*CO), 20.5, 19.5 (CH*Me*₂).

4.1.1.2. *tert*-Butyl (2*R*)-2-(*N*-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)biphenyl-4-ylsulfonamido)-3-methylbutanoate (20). The Mitsunobu reaction was conducted following the general procedure, starting from a solution of compound **17** (50.0 mg, 0.144 mmol) in dry THF (2.90 mL), the biphenylsulfonamide **18** (83.9 mg, 0.215 mmol), PPh_3 (118.0 mg, 0.502 mmol), and DMEAD (118 mg, 0.502 mmol). The reaction mixture was stirred at room temperature for 46 h. After work-up, the crude residue (380 mg) was purified by flash chromatography (*n*-hexane-EtOAc 6:4 + 0.01% Et_3N) affording derivative **20** (41 mg, 40% yield) as white foam. ^{1}H NMR (250.12 MHz, $CDCl_3$ δ 8.01 (m, 2H, 2×Ar-H), 7.68 (m, 2H, 2×Ar-H), 7.57 (m, 2H, 2×Ar-H), 7.51-7.26 (m, 3H, 3×Ar-H), 5.85 (dd, 1H, $J_{2,3}$=9.3 Hz, $J_{3,4}$=8.6 Hz, H-3), 5.42 (d, 1H, $J_{1,2}$=9.7 Hz, H-1), 5.09 (dd, 1H, H-2), 5.14 (dd, 1H, $J_{4,5}$=9.7 Hz, H-4), 4.13 (dd, 1H, $J_{6a,6b}$=12.0 Hz, $J_{5,6b}$=5.6 Hz, H-6b), 3.96 (m, 1H, H-6a), 3.64 (ddd, 1H, $J_{5,6a}$=2.6 Hz, H-5), 3.94 (d, 1H, *J*=10.2 Hz, *CH*NSO_2), 2.03, 2.01, 1.98, 1.80 (4s, each 3H, 4×*Me*COO), 1.81 (m, 1H, *CH*Me_2), 1.35 (s, 9H, C*Me₃*), 0.97 (d, 3H, J_{vic}=6.7 Hz, CH*Me*₂), 0.92 (d, 3H, J_{vi} =6.7 Hz, CH*Me*₂). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 171.1-169.8 (4×C=O), 146.4 (Ar-*C*-SO_2), 140.0, 139.9 (2×Ar-*C*), 129.1-128.3 (Ar-*C*H), 85.6 (C-1), 82.7 (*C*Me_3), 75.7 (C-2), 74.6 (C-3), 70.2 (C-5), 68.8 (C-4), 67.3 (*CH*NSO_2), 63.0 (C-6), 31.3 (*CH*Me_2), 28.5 (C*Me₃*), 21.2 (4×*Me*CO), 20.6, 20.4 (CH*Me*₂).

4.1.1.3. *tert*-Butyl (2*R*)-3-methyl-2-(*N*-(((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4-diacetoxy-5-acetamido-6-methoxytetrahydro-2H-pyran-2-yl)methyl)biphenyl-4-ylsulfonamido)butanoate (23). The

Mitsunobu reaction was conducted following the general procedure, starting from a solution of compound **21** (100 mg, 0.3122 mmol) in dry THF (6 mL), the biphenylsulfonamide **18** (122 mg, 0.3122 mmol), PPh_3 (287 mg, 1.09 mmol), and DIAD (0.21 mL). The reaction mixture was stirred at room temperature for 48 h. After work-up, the crude (925 mg) was purified by flash chromatography using an Isolute Flash Si II cartridge (Petroleum Ether- EtOAc in gradient from 2:1 to 1:2) affording derivative **23** (79 mg, 40% yield), as syrup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84-7.81 (m, 2H, 2× Ar-*H*), 7.69-64 (m, 2H, 2× Ar-*H*), 7.56-7.53 (m, 2H, 2× Ar-*H*), 7.49-7.44 (m, 3H, 3× Ar-H), 5.48 (d, 1H, $J_{2,NH}$ = 8.2 Hz, N*H*Ac), 5.18 (dd, 1H, $J_{2,3}$=10.8 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 4.84 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 4.42 (d, 1H, $J_{1,2}$=8.4 Hz, H-1),) 4.15 (ddd, $J_{5,6a}$=1.2 Hz, $J_{5,6b}$=8.4 Hz, 1H, H-5), 4.02 (dd, 1H, H-2), 3.96 (d, 1H, *J*=10.0 Hz, *CH*NSO_2), 3.74 (dd, $J_{6a,6b}$=16.1 Hz, 1H, H-6a), 3.49 (s, 3H, OMe), 3.41 (dd, 1H, H-6b) 2.15, 2.03, 1.96 (3s, each 3H, 2×*Me*COO, *Me*CON), 2.04-2.02 (m, 1H, Me_2*CH*), 1.19 (s, 9H, Me_3C), 1.09 (d, 3H, J_{vic}=6.4 Hz, Me_2CH), 0.93 (d, 3H, J_{vic} = 6.4 Hz, Me_2CH).

4.1.1.4. *tert*-Butyl (2*R*)-3-methyl-2-(*N*-(((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-triacetoxy-6-methoxytetrahydro-2H-pyran-2-yl)methyl)biphenyl-4-ylsulfonamido)butanoate (24). The Mitsunobu reaction was conducted following the general procedure, starting from a solution of compound **22** (50.0 mg, 0.144 mmol) in dry THF (2.90 mL), the biphenylsulfonamide **18** (83.9 mg, 0.215 mmol), PPh_3 (118.0 mg, 0.502 mmol), and DMEAD (118 mg, 0.502 mmol). The reaction mixture was stirred at room temperature for 46 h. After the treatment, the crude residue (380 mg), was purified by flash chromatography (*n*-hexane-EtOAc 6:4 + 0.01% Et_3N) affording derivative **24** (41 mg, 40% yield) as a white foam. ^{1}H NMR (250.12 MHz, $CDCl_3$): δ 8.01 (m, 2H, 2×Ar-*H*), 7.68 (m, 2H, 2×Ar-*H*), 7.57 (m, 2H, 2×Ar-*H*), 7.51-7.26 (m, 3H, 3×Ar-*H*), 5.85 (dd, 1H, $J_{2,3}$=9.3 Hz, $J_{3,4}$=8.6 Hz, H-3), 5.42 (d, 1H, $J_{1,2}$=9.7 Hz, H-1), 5.09 (dd, 1H, H-2), 5.14 (dd, 1H, $J_{4,5}$=9.7 Hz, H-4), 4.13 (dd, 1H, $J_{6a,6b}$=12.0 Hz, $J_{5,6b}$=5.6 Hz, H-6b), 3.96 (m, 1H, H-6a), 3.64 (ddd, 1H, $J_{5,6a}$=2.6 Hz, H-5), 3.94 (d, 1H, *J*=10.2 Hz, *CH*NSO_2), 2.03, 2.01, 1.98, 1.80 (4s, each 3H, 4×*Me*CO), 1.81 (m, 1H, *CH*Me_2), 1.35 (s, 9H, CMe_3), 0.97 (d, 3H, J_{vic}=6.7 Hz, CHMe_2), 0.92 (d, 3H, J_{vic}=6.7 Hz, CHMe_2). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 171.1-169.8 (4×*C*=O), 146.4 (Ar-*C*-SO_2), 140.0, 139.9 (2×Ar-*C*), 129.1-128.3 (Ar-*C*H), 85.6 (C-1), 82.7 (*C*Me_3), 75.7 (C-2), 74.6 (C-3), 70.2 (C-5), 68.8 (C-4), 67.3 (*CH*NSO_2), 63.0 (C-6), 31.3 (*CH*Me_2), 28.5 (CMe_3), 21.2 (4×*Me*CO), 20.6, 20.4 (CHMe_2).

4.1.2. 2,3,4,6-Tri-*O*-acetyl-β-D-glucopyranosyl azide (25).

To a solution of the commercial 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1.00 g, 2.43 mmol) in dry DMF (3 mL) 205 mg (3.16 mmol) of NaN_3 were added and the mixture was stirred for

two days. The solvent was removed *in vacuo* and the residue was taken up with EtOAc and water and the aqueous phase was extracted with EtOAc (3×20 mL). The organic phases were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure affording the pure azide **25** (762 mg, quantitative yield) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.22 (dd, $J_{2,3}$=9.4 Hz, $J_{3,4}$=9.6 Hz, 1H, H-3), 5.10 (dd, $J_{4,5}$=10.4 Hz, 1H, H-4), 4.94 (dd, $J_{1,2}$=8.9 Hz, 1H, H-2), 4.64 (d, 1H, H-1), 4.28 (dd, $J_{5,6b}$=4.8 Hz, $J_{6a,6b}$=12.4 Hz, 1H, H-6b), 4.17 (dd, $J_{5,6a}$=2.3 Hz, 1H, H-6a), 3.79 (ddd, 1H, H-5), 2.10, 2.07, 2.03, 2.01 (3s, each 3H, 4 × *Me*CO).

4.1.3. 2,3,4,6-Tri-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (27).

To solution of azide **25** (762 mg, 2.04 mmol) in amixture 20:1 THF-H_2O (6.45 mL), commercial PPh_3 (1.08 g) supported on resin (3 mmol/g) was added and the mixture was stirred at room temperature until the starting compound was completely reacted. After filtration over a small layer of Celite the solution was concentrated under reduced pressure and the crude residue was constituted exclusively by amine **26** (254.5 mg) as clear syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.24 (dd, $J_{2,3}$=9.4 Hz, $J_{3,4}$=9.6 Hz, 1H, H-3), 5.05 (dd, $J_{4,5}$=10.0 Hz, 1H, H-4), 4.90 (dd, $J_{1,2}$=9.2 Hz, 1H, H-2), 4.30 (bd, 1H, H-1), 4.23 (dd, $J_{5,6b}$=4.4 Hz, $J_{6a,6b}$=12.0 Hz, 1H, H-6b), 4.11 (dd, $J_{5,6a}$=2.4 Hz, 1H, H-6a), 3.73 (ddd, 1H, H-5), 2.10, 2.09, 2.03, 2.01 (4s, each 3H, 4 × *Me*CO).
Without further purification, a solution of amine **26** (254 mg) in dry CH_2Cl_2 (10 mL) was treated with 2-pyridyl-thiocarbonate (DPT, 473 mg, 2.04 mmol). After stirring at room temperature overnight, the mixture was diluted with CH_2Cl_2 (20 mL) and water (20 mL) and then extracted with CH_2Cl_2 (4×20 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (*n*-hexane/EtOAc 10:1) and trituration of the crude with hexane geve the isothiocianate **27** (119 mg, 15% yield from **25**) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.20 (dd, $J_{2,3}$=9.2 Hz, $J_{3,4}$=9.4 Hz, 1H, H-3), 5.11 (dd, 1H, $J_{4,5}$=10.1 Hz, H-4), 5.09 (dd, $J_{1,2}$=8.9 Hz, 1H, H-2), 5.01 (d, 1H, H-1), 4.24 ($J_{5,6b}$=4.8 Hz, $J_{6a,6b}$=12.5 Hz, 1H, H-6b), 4.14 ($J_{5,6a}$=2.3 Hz, 1H, H-6a), 3.74 (ddd, 1H, H-5), 2.11, 2.10, 2.03, 2.02, (4s, each 3H, 4×*Me*CO).

4.1.4. *tert*-Butyl (2*R*)-3-methyl-2-(*N*-(2-(3-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)thioureido)ethyl)biphenyl-4-ylsulfonamido)butanoate (29).

The glucose isothiocyanate **27** (135 mg, 0.3467 mmol) was dissolved in dry 4:1 CH_2Cl_2/DMF mixture (10 mL) and the solution of amine **18** (173 mg, 0.3152 mmol) in dry CH_2Cl_2/DMF 4:1 (10 mL)

containing Et_3N (0.043 mL) was added dropwise. The reaction mixture was stirred at 60 °C until TLC analysis (EtOAc) revealed the complete disappearance of the amines (12 h) and concentrated under reduced pressure. The purification of crude product by flash chromatography (Petroleum Ether/EtOAc 3:1) using an Isolute Flash Si II cartridge afforded pure **29** (145 mg, 83% yield) as a white foam. ^{1}H NMR (CD_3CN, 400 MHz): δ 7.91(m, 2H, 2×Ar-*H*), 7.81(m, 2H, 2×Ar-*H*), 7.70 (m, 2H, 2×Ar-*H*), 7.55-7.47 (m, 3H, 2×Ar-*H*), 7.03 (bs, 1H, N*H*C=S), 6.84 (bs, 1H, N*H*C=S), 5.36 (dd, $J_{2,3}$=9.4 Hz, $J_{3,4}$=9.6 Hz, 1H, H-3), 5.04 (dd, $J_{4,5}$=9.8 Hz, 1H, H-4), 5.02 (dd, $J_{1,2}$=7.2 Hz, 1H, H-2), 4.21 (dd, $J_{6a,6b}$=12.3 Hz, $J_{5,6b}$=4.9Hz 1H, H-6b), 4.21 (dd, $J_{5,6a}$=2.7 Hz 1H, H-6a), 4.08 (d, 1H, H-1), 3.92 (ddd, 1H, H-5), 3.90 (d, J_{vic}=10.6 Hz, 1H, *CH*NSO2), 3.89-3.80 (m, 3H, *CH2*NHCS, *CH2*NSO2), 3.40 (m, 1H, *CH2*NSO2), 2.22 (m, 1H, C*HMe2*), 2.02, 2.01, 2.00, 1.98 (4s, each 3H, 4×*Me*CO). 1.25 (s, 9H, C*Me3*), 1.03 (d, 3H, J_{vic}= 6.6 Hz, CH*Me2*), 0.96 (d, 3H, *Jvic*= 6.8 Hz, CH*Me2*).

4.1.5 *tert*-Butyl (2*R*)-(*N*-(2-isothiocyanatoethyl)-[1,1'-biphenyl]-4-ylsulfonamido)-3-methylbutanoate (30).

To a solution of trifluoroacetate salt **28** (300 mg, 0.549 mmol) in dry CH_2Cl_2 (9.0 mL) 2-pyridyl-thiocarbonate (DPT, 127 mg, 0.549 mmol) was added. The reaction mixture was stirred at room temperature overnight under nitrogen atmosphere. The mixture was diluted with CH_2Cl_2 (20 mL) and water (20 mL) and then extracted with CH_2Cl_2 (4×20 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification of the crude by flash chromatography using an Isolute Flash Si II cartridge (*n*-hexane/EtOAc 5:1) afforded isothyocianate derivative **30** (202 mg, 78% yield) as a white foam. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.90 (m, 2H, 2×Ar-*H*), 7.72 (m, 2H, 2×Ar-*H*), 7.57 (m, 2H, 2×Ar-*H*), 7.50-7.40 (m, 3H, 3×Ar-*H*), 3.97 (d, J_{vic}=10.3 Hz, 1H, *CH*NSO2), 4.03-3.96, 3.94-3.76 (2m, 3H, C*H2*NHCS, *CH2*NSO2), 3.54 (ddd, J_{gem}=14.6 Hz, J_{vic}=8.8 Hz, J_{vic}=5.3 Hz, 1H, *CH2*NSO2), 2.03 (m, 1H, C*H*Me2), 1.26 (s, 9H, C*Me3*), 1.03 (d, 3H, J_{vic}=6.6 Hz, CH*Me2*), 0.98 (d, 3H, J_{vic}=6.6 Hz, CH*Me2*).

4.1.6. Methyl 2,3,4-tri-*O*-acetyl-6-amino-6-deoxy-β-D-glucopyranoside (32).

To a solution of the azide **31** (50 mg, 0.145 mmol) in a mixture of DCM:H_2O:MeOH 3:1:0.5 (17 mL), 109 mg di Pd/C 10%.were added. The reaction was stirred overnight under hydrogen atmosphere. After filtration over a small layer of Celite, the solution was concentrated under reduced pressure and the residue was constituted exclusively by amine **32** as clear syrup. ^{1}H NMR (400 MHz, CD_3OD): δ 5.31 (dd, $J_{2,3}$=9.4 Hz, $J_{3,4}$=9.6 Hz, 1H, H-3), 4.95 (m, 2H, H-2, H-4), 4.65 (d, $J_{1,2}$=8.0 Hz, 1H, H-1), 3.94

(ddd, 1H, $J_{4,5}$=10.3 Hz $J_{5,6a}$=3.0 Hz, $J_{5,6b}$=8.4 Hz H-5), 3.55 (s, 3H, OMe); 3.24 (dd, $J_{6a,6b}$=13.6 Hz 1H, H-6a), 3.05 (dd, 1H, H-6b), 2.06, 2.02, 1.98 (3s, each 3H, 3 × *Me*CO). ^{13}C NMR (100.62 MHz, CD_3OD) δ: 171.7, 171.4, 171.1 (3×C=O), 102.8 (C-1), 73.6 (C-3), 72.5 (C-5), 71.3, 71.1 (C-2, C-4), 57.8 (OMe), 41.3 (C-6), 20.5 (3×*Me*CO).

4.1.7. *tert*-Butyl (2*R*)-3-methyl-2-(*N*-(2-(3-(((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-triacetoxy-6-methoxytetrahydro-2H-pyran-2-yl)methyl)thioureido)ethyl)biphenyl-4-ylsulfonamido)butanoate (33).

The isothiocyanate **30** (40 mg, 0.08 mmol) was dissolved in dry 4:1 CH_2Cl_2/DMF mixture (1 mL) and the solution of the C-6 glucosamine **32** (25 mg, 0.07 mmol) in dry CH_2Cl_2/DMF 4:1 (1 mL) containing Et_3N (0.01 mL) was added dropwise. The reaction mixture was stirred at 60 °C until TLC analysis (EtOAc) revealed the complete disappearance of the amines (12 h) and concentrated under reduced pressure. Flash chromatographic purification over silica gel of the crude product (*n*-hexane/EtOAc 2:1) using an Isolute Flash Si II cartridge afforded pure **33** (29 mg, 47 % yield) as a clear syrup. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.88 (m, 2H, 2×Ar-*H*); 7.71 (m, 2H, 2×Ar-*H*); 7.58 (m, 2H, 2×Ar-*H*); 7.56-7.41 (m, 3H, 3×Ar-*H*); 5.19 (dd, $J_{2,3}$=9.6 Hz, $J_{3,4}$=9.2 Hz, 1H, H-3), 5.00-4.94 (m, 2H, H-2, H-4), 4.44 (d, $J_{1,2}$=8.0 Hz, 1H, H-1), 3.90 (d, *J*=10.0 Hz, 1H, *CH*NSO_2), 4.10-3.67 (m, 5H, H-5, H-6b, CH_2NHCS, CH_2NSO_2), 3.49 (s, 3H, OMe); 3.55-3.42 (m, 2H, H-6a, CH_2NSO_2), 2.11 (m, 1H, C*H*Me_2), 2.06, 2.04, 1.98 (3s, each 3H, 3×*Me*CO), 1.25 (s, 9H, CMe_3), 0.99 (d, 3H, J_{vic}= 6.4 Hz, CHMe_2), 0.95 (d, 3H, J_{vic}=6.5 Hz, CHMe_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.3 (C=S), 170.4, 170.3, 170.0, 169.5 (4×C=O), 146.2 (Ar-*C*-SO_2), 139.3, 138.0 (2×Ar-*C*), 129.2.-127.4 (Ar-*C*H), 101.7 (C-1), 82.6 (Me_3*C*O), 72.8 (C-3), 71.4, 71.3 (C-5, C-2), 70.0 (C-4), 57.3 (OMe), 66.8 (*CH*NSO_2), 42.1-43.5 (C-6, CH_2NHCS, CH_2NSO_2), 29.8 (C*H*Me_2), 27.9 (CMe_3), 20.9, 20.8, 20.7 (3×*Me*CO), 20.2, 19.3 (CHMe_2).

4.1.8 General procedure for the synthesis of carboxylic acids 4, 5, 8, 9, 12 and 14. The appropriate ester (**19**, **20**, **23**, **24**, **29** or **33**, 0.1 mmol) was dissolved in CH_2Cl_2 (1.7 mL), treated with CF_3COOH (0.6 mL) and stirred at 0 °C for 1 h. After 5-24 h at room temperature, TLC analysis (EtOAc) revealed the complete disappearance of the starting material and the formation of a more retained product. The solution was coevaporated with toluene (4×10 mL) under reduced pressure and trituration of the crude product with Et_2O afforded pure carboxylic acids **4**, **5**, **8**, **9**, **12** and **14.**

4.1.8.1. (2*R*)-2-(*N*-((2*R*,3*R*,4*S*,5*S*,6*R*)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)biphenyl-4-ylsulfonamido)-3-methylbutanoic acid (4). The title compound was prepared

from ester **19** following the general procedure. Purification by trituration with *n*-hexane afforded pure **4** (52 mg, 89 % yield) as a white solid; R_f 0.46 (*n*-hexane-EtOAc 1:9); mp. 86-90 °C; ^{1}H NMR (250 MHz, CD_3CN-CD_3OD): δ 8.01 (m, 2H, 2×Ar-*H*), 7.77 (m, 2H, 2×Ar-*H*), 7.58-7.44 (m, 6H, 5×Ar-*H*, N*H*Ac), 5.24-5.14 (m, 2H, H-1, H-3), 5.07-4.89 (m, 2H, H-2, H-4), 4.16 (m, 1H, H-6b), 4.03-3.89 (m, 2H, H-6a, *CH*NSO_2), 3.82 (m, 1H, H-5), 2.15 (m, 1H, *CH*Me_2), 1.95, 1.94, 1.93 (3s, each 3H, 3×*Me*CO), 1.85 (s, 3H, *Me*CON), 0.90 (d, 3H, J_{vic}=6.7 Hz, Me_2CH), 0.67 (d, 3H, J_{vic}=6.7 Hz, CHMe_2). ^{13}C NMR (62.9 MHz , CD_3CN-CD_3OD): δ 171.9, 171.8, 171.7, 171.6, 171.1 (5×C=O), 146.7 (Ar-*C*-SO_2), 140.1, 139.9 (2×Ar-*C*), 130.3-128.2 (Ar-*C*H), 85.7 (C-1), 76.0 (C-3), 74.8 (C-5), 69.6 (C-4), 64.5 (*CH*NSO_2), 63.6 (C-6), 54.0 (C-2), 30.5 (*CH*Me_2), 23.3 (*Me*CON), 21.3 (3×*Me*COO), 20.3, 20.2 (CHMe_2). Elemental Analysis for $C_{31}H_{38}N_2O_{12}S$ calculated: % C, 56.18; % H, 5.78; % N 4.23; found: % C, 56.23; % H, 5.81; % N 4.20.

4.1.8.2. (2*R*)-2-(*N*-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)biphenyl-4-ylsulfonamido)-3-methylbutanoic acid (5). The title compound was prepared from ester **20** following the general procedure as a white solid (11 mg, 78 % yield). R_f 0.53 (*n*-hexane-EtOAc 1:9); mp. 88-90 °C; ^{1}H NMR (250 MHz, CD_3CN-CD_3OD): δ 7.99 (m, 2H, 2×Ar-*H*), 7.69-7.58 (m, 4H, 4×Ar-*H*), 7.51-7.31 (m, 3H, 3×Ar-*H*), 5.65-5.50 (m, 2H, H-1, H-3), 5.10-4.91 (m, 2H, H-2, H-4), 4.13 (m, 1H, H-6b), 4.05-3.70 (m, 3H, H-6a, H-5, *CH*NSO_2), 2.01, 1.99, 1.95, 1.90 (4s, each 3H, 4×*Me*CO), 1.85 (m, 1H, *CH*Me_2), 0.95 (d, 3H, J_{vic}=6.7 Hz, CHMe_2), 0.71 (d, 3H, J_{vic}=6.7 Hz, CHMe_2). ^{13}C NMR (CD_3CN-CD_3OD 62.9 MHz): δ 171.4, 171.2, 170.5, 170.3, 170.2 (5×C=O), 146.4 (Ar-*C*-SO_2), 139.5, 138.2 (2×Ar-*C*), 129.5-127.6 (Ar-*C*H), 85.3 (C-1), 75.2 (C-2), 74.4 (C-3), 70.5 (C-5), 68.6 (C-4), 67.4 (*CH*NSO_2), 62.8 (C-6), 30.1 (*CH*Me_2), 20.9 (4×*Me*CO), 21.7, 21.0 (CHMe_2). Elemental Analysis for $C_{31}H_{37}NO_{13}S$ calculated: % C, 56.10; % H, 5.62; % N 2.11; found: % C, 56.15; % H, 5.68; % N 2.10.

4.1.8.3. (2*R*)-3-methyl-2-(*N*-(((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4-diacetoxy-5-acetamido-6-methoxytetrahydro-2H-pyran-2-yl)methyl)biphenyl-4-ylsulfonamido)butanoic acid (8). The title compound was prepared from ester **23** following the general procedure. Purification by flash cromathography using an Isolute Silica II cartridge ($CHCl_3$-MeOH 50:1) afforded pure **8** (60 mg, 52% yield) as a white solid; R_f 0.37 ($CHCl_3$-MeOH 20:1); mp. 100-102 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.87 (bs, 1H, COO*H*), 7.91 (m, 2H, 2× Ar-*H*), 7.81-7.57 (m, 4H, 3×Ar-*H, NH*Ac), 7.61-7.50 (m, 3H, 3×Ar-*H*), 7.45 (m, 1H, Ar-*H*), 5.10 (dd, 1H, $J_{2,3}$=10.3 Hz, $J_{3,4}$=9.6 Hz, H-3), 4.64 (dd, $J_{4,5}$=9.7 Hz, 1H, H-4), 4.47 (d, $J_{1,2}$=8.4 Hz, 1H, H-1), 3.99 (m, 1H, H-2), 3.93 (d, *J*=10 Hz, *CH*NSO_2), 3.74 (ddd, $J_{5,6a}$=1.2 Hz, $J_{5,6b}$=8.6 Hz,

1H, H-5), 3.52 (dd, 1H, $J_{6a,6b}$=16.4 Hz, H6b), 3.32 (s, 3H, OMe), 3.21 (dd, 1H, H-6a), 1.93 (m, 1H, Me$_2$*CH*), 2.11, 1.93 (2s, each 3H, 2×*Me*CO), 1.76 (s, 3H, *Me*CONH), 1.01 (d, 3H, J_{vic}=6.5 Hz, CH*Me$_2$*), 0.92 (d, 3H, J_{vic}=6.5 Hz, CH*Me$_2$*). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.7, 170.3, 170.1, 169.6 (4×C=O), 145.0 (Ar-*C*-SO$_2$), 138.6, 137.1 (2×Ar-*C*), 132.0-127.6 (Ar-*C*H), 101.7 (C-1), 73.8, 73.1 (C-2, C-3), 71.1 (C-4), 66.4 (*CH*NSO$_2$), 56.8 (OMe), 53.3 (C-2), 47.2 (C-6), 28.7 (*CH*Me$_2$), 23.2 (*Me*CON), 21.0,19.6 (CH*Me$_2$*), 20.8, 20.9 (2×*Me*CO). Elemental Analysis for $C_{30}H_{38}N_2O_{11}S$ calculated: % C, 56.77; % H, 6.03; % N 4.41; found: % C, 56.81; % H, 5.10; % N 4.40.

4.1.8.4. (2*R*)-3-methyl-2-(*N*-(((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-triacetoxy-6-methoxytetrahydro-2H-pyran-2-yl)methyl)biphenyl-4-ylsulfonamido)butanoic acid (9). The title compound was prepared from ester **24** following the general procedure. Purification by flash cromathography (*n*-hexane-EtOAc 45:55) followed by trituration with Et$_2$O afforded pure **9** (56 mg, 89% yield) as a white solid; R$_f$ 0.51 (*n*-hexane-EtOAc 3:7); mp. 77-80 °C; ^{1}H NMR (250 MHz, CD$_3$CN-D$_2$O): δ 7.85-7.70 (m, 4H, 4×Ar-*H*), 7.66 (m, 2H, 2×Ar-*H*), 7.55-7.39 (m, 3H, 3×Ar-*H*), 5.24 (dd, 1H, $J_{2,3}$=9.9 Hz, $J_{3,4}$=9.3 Hz, H-3), 4.83 (dd, 1H, $J_{1,2}$=8.0 Hz, H-2), 4.80 (dd, 1H, $J_{4,5}$=10.0 Hz, H-4), 4.49 (d, 1H, H-1), 4.12 (ddd, 1H, $J_{5,6a}$=2.0 Hz, $J_{5,6b}$=8.5 Hz, H-5), 3.98 (d, 1H, *J*=10.2 Hz, *CH*NSO$_2$), 3.57 (dd, 1H, $J_{6a,6b}$=16.3 Hz, H-6b), 3.40 (s, 3H, OMe), 3.33 (dd, 1H, H-6a), 2.15 (m, 1H, *CH*Me$_2$), 2.09, 1.99, 1.95 (3s, each 3H, 3×*Me*CO), 1.01 (d, 3H, J_{vic}=6.5 Hz, CH*Me$_2$*), 0.95 (d, 3H, J_{vic}=6.5 Hz, CH*Me$_2$*). ^{13}C NMR (62.9 MHz, CD$_3$CN-D$_2$O): δ 171.7, 170.7, 170.6, 170.1 (4×C=O), 146.2 (Ar-*C*-SO$_2$), 139.6, 139.7 (2×Ar-*C*), 129.8-127.9 (Ar-*C*H), 102.0 (C-1), 74.4 (C-5), 73.1 (C-3), 71.6 (C-2), 71.3 (C-4), 66.6 (*CH*NSO$_2$), 57.8 (OMe), 47.7 (C-6), 29.3 (*CH*Me$_2$), 20.8 (3×*Me*COO), 21.0, 19.4 (CH*Me$_2$*). Elemental Analysis for $C_{30}H_{37}NO_{12}S$ calculated: % C, 56.68; % H, 5.87; % N 2.20; found: % C, 56.72; % H, 5.90; % N 2.17.

4.1.8.5. (2*R*)-3-methyl-2-(*N*-(2-(3-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)thioureido)ethyl)biphenyl-4-ylsulfonamido)butanoic acid (12). The title compound was prepared from ester **29** following the general procedure. Purification by flash cromathography using an Isolute Flash Si II cartridge (CHCl$_3$-MeOH 20:1) afforded pure **12** (41 mg, 32% yield) as a white solid; mp. 140-143 °C. ^{1}H NMR (250 MHz, CD$_3$CN): δ 7.85 (m, 2H, 2×Ar-*H*), 7.79 (m, 2H, 2×Ar-*H*), 7.68 (m, 2H, 2×Ar-*H*), 7.64-7.51 (m,3H, 3×Ar-*H*), 7.13 (bs, 1H, CH$_2$N*H*CS), (bd, $J_{2,3}$=8.8 Hz, 1H, CHN*H*CS), 5.61-5.68 (m, 1H, H-1), 5.34 (dd, $J_{2,3}$=9.6 Hz, $J_{3,4}$=9.5 Hz ,1H, H-3), 5.02-4.91 (m, 2H, H-2, H-4), 4.20 (dd, $J_{5,6b}$=4.8 Hz 1H, $J_{6a,6b}$=12.4 Hz, H-6b), 4.06 (dd, $J_{5,6a}$=2.1 Hz 1H, H-6a), 3.99 (d, *J*=10.4 Hz 1H, *CH*NSO$_2$), 4.00-3.70 (m, 4H, H-5, *CH$_2$*NHCS,*CH$_2$*NSO$_2$), 3.34 (m, 1H, *CH$_2$*NHCS), 2.02 (m, 1H, *CH*(Me)$_2$), 1.98, 1.97, 1.96, 1.95 (4s,

each 3H, CH_3CO), 0.92 (bd, 6H, CH(*Me*)$_2$). ^{13}C NMR (69.12 MHz, CD_3CN): δ 184.9 (C=S), 172.2, 171.3, 170.8, 170.5 (4 × C=O) 146.2 (Ar-*C*-SO_2), 139.8, 138.3, (2×Ar-*C*) 130.0-128.1 (Ar-*C*H), 82.7 (C-1), 73.6, 73.5, 71.4, (C-2, C-3, C-5), 69.8 (C-4), 66.7 (*C*HNSO_2), 62.7 (C-6), 45.5, 44.3 (*C*H_2NHCS, *C*H_2NSO_2), 29.17 (*CH*Me_2), 20.9, (4 × *Me*CO), 20.4, 19.5 (CH*Me*$_2$). Elemental Analysis for $C_{34}H_{43}N_3O_{13}S_2$ calculated: % C, 53.32; % H, 5.66; % N 5.49; found: % C, 53.42; % H, 5.70; % N 5.51.

4.1.8.6. (2*R*)-3-methyl-2-(*N*-(2-(3-(((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-triacetoxy-6-methoxytetrahydro-2H-pyran-2-yl)methyl)thioureido)ethyl)biphenyl-4-ylsulfonamido)butanoic acid (14). The title compound was prepared from ester **33** following the general procedure. Purification by flash cromathography using Isolute Flash Si II cartridge ($CHCl_3$) afforded pure **14** (19.5 mg, 52.5% yield) as a white solid; mp. 112-115 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.95 (m, 2H, 2×Ar-*H*), 7.81 (m, 2H, 2×Ar-*H*), 7.68 (m, 2H, 2×Ar-*H*), 7.49 (m, 2H, 2×Ar-H), 7.41 (m, 1H, Ar-*H*), 5.22 (dd, $J_{2,3}$=9.6 Hz, $J_{3,4}$=9.4 Hz, 1H, H-3), 4.92-4.86 (m, 2H, H-2, H-4), 4.53 (d, $J_{1,2}$=8.0 Hz, 1H, H-1), 4.02 (d, *J*=10.4 Hz, 1H, *CH*NSO_2), 3.82-3.76 (m, 5H, H-5, H-6a, H-6b, *CH*$_2$NSO_2,*CH*$_2$NHCS), 3.64 (m, 1H, *CH*$_2$NSO_2), 3.47 (s, 3H, OMe); 2.25 (m, 1H, *CH*Me_2), 2.04, 2.00, 1.94 (3s, each 3H, MeCO), 1.04 (d, J_{vic}=6.4 Hz, 3H, CH*Me*$_2$); 0.99 (d, J_{vic}=6.8 Hz, 3H, CH*Me*$_2$). ^{13}C NMR (100 MHz, CD_3OD): δ 183.1(C=S), 173.7, 171.6, 171.5, 171.2 (4×C=O), 147.0, (Ar-*C*-SO_2), 140.6, 138.9 (2×Ar-*C*), 130.1-128.3 (Ar-*C*H), 102.6 (C-1), 74.4 (C-3), 73.7 (C-5), 72.8, (C-2), 71.1 (C-4), 67.9 (*CH*NSO_2), 57.4 (OMe), 44.1-46.5 (C-6, *CH*$_2$NHCS, *CH*$_2$NSO_2), 29.8 (*CH*Me_2), 20.9, 20.7, 20.3 (3×*Me*CO), 20.6, 19.8 (CH*Me*$_2$). Elemental Analysis for $C_{33}H_{43}N_3O_{12}S_2$ calculated: % C, 53.72; % H, 5.87; % N 5.70; found: % C, 53.80; % H, 5.90; % N 5.65.

4.1.9. General procedure for the synthesis of deprotected carboxylic acids 6, 7, 10, 11, 13 and 15.

A solution of the appropriate protected carboxylic acid (**4**, **5**, **8**, **9**, **12** and **14**, 0.1 mmol) in MeOH (1 mL) was treated with NH_3-MeOH 7N (1 mL) and the solution was stirred at room temperature until the starting compound was completely reacted (TLC, $CHCl_3$/MeOH 8:2, 20-24 h). The solution was coevaporated with toluene (4×10 mL) under reduced pressure and the crystallization (MeOH/EtOAc) or trituration of crude product with Et_2O afforded pure carboxylic acids **6**, **7**, **10**, **11**, **13** and **15.**

4.1.9.1. (2*R*)-2-(*N*-((2*R*,3*R*,4*S*,5*S*,6*R*)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)biphenyl-4-ylsulfonamido)-3-methylbutanoic acid (6). The title compound was prepared from the protected carboxylic acid **4** following the general procedure. Trituration with Et_2O afforded pure **6** (94% yield) as a white solid; mp. 151-155 °C; ^{1}H NMR (250 MHz, CD_3OD): δ 7.94-

7.31 (m, 7H, 7×Ar-*H*), 7.25-7.08 (m, 3H, 2×Ar-*H*, N*H*Ac), 5.08 (m, H, H-1), 4.10-3.12 (m, 7H, H-2, H-3, H-4, H-5, H-6a, H-6b, *CH*NSO_2), 1.95 (m, 1H, Me_2*CH*), 1.83 (s, 3H, *Me*CON), 1.05-0.81 (m, 6H, *Me*$_2$CH). ^{13}C NMR (62.9 MHz, CD_3OD): δ 172.1 (C=O), 146.6 (Ar-C-SO_2), 141.3, 140.5 (2×Ar-*C*), 130.1-128.2 (Ar-*C*H), 86.3 (C-1), 80.4, 77.7 (C-3, C-5), 71.7 (C-4), 62.6 (C-6), 56.5 (C-2), 65.4 (*CH*NSO_2), 30.7 (*CH*Me_2), 21.9 (*Me*CON), 20.7, 20.2 (*Me*$_2$CH). Elemental Analysis for $C_{25}H_{32}N_2O_9S$ calculated: % C, 55.96; % H, 6.01; % N 5.22; found: % C, 55.98; % H, 6.07; % N 5.20.

4.1.9.2. (2*R*)-2-(*N*-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)biphenyl-4-ylsulfonamido)-3-methylbutanoic acid (7). The title compound was prepared from the protected carboxylic acid **5** following the general procedure. Trituration with Et_2O afforded pure **7** (94% yield) as a white solid; mp. 127-130 °C; ^{1}H NMR (250 MHz, CD_3OD): δ 8.08 (m, 2H, 2×Ar-H), 7.91-7.67 (m, 4H, 4×Ar-H), 7.79-7.39 (m, 3H, 3×Ar-H), 4.20-3.21 (m, 12H, H-1, H-2, H-3, H-4, H-5, H-6a, H-6b, *CH*NSO_2, 4×OH), 2.15 (m, 1H, *CH*Me_2), 0.99-0.75 (m, 6H, CH*Me*$_2$). ^{13}C NMR (62.9 MHz, CD_3OD): δ 174.1 (C=O), 146.1 (Ar-C-SO_2), 140.6, 140.3 (2×Ar-*C*), 129.6-127.7 (Ar-*C*H), 88.6 (C-1), 80.2, 79.7, 72.6, 708 (C-2, C-3, C-4, C-5), 67.9 (*CH*NSO_2), 62.4 (C-6), 30.3 (*CH*Me_2), 21.7, 20.9 (CH*Me*$_2$). Elemental Analysis for $C_{23}H_{29}NO_9S$ calculated: % C, 55.75; % H, 5.90; % N 2.83; found: % C, 55.81; % H, 5.95; % N 2.85.

4.1.9.3. (2*R*)-3-methyl-2-(*N*-(((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4-dihydroxy-5-acetamido-6-methoxytetrahydro-2H-pyran-2-yl)methyl)biphenyl-4-ylsulfonamido)butanoic acid (10). The title compound was prepared from the protected carboxylic acid **8** following the general procedure. Purification by flash cromathography using Isolute Flash Si II cartridge ($CHCl_3$-MeOH 20:1) afforded pure **10** (12 mg, 35% yield) as a white solid. Mp. 116-117 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 8.05 (d, $J_{2,NH}$=9.2 Hz, N*H*Ac), 7.96 (m, 2H, 2×Ar-H), 7.80 (m, 2H, 2×Ar-H), 7.68 (m, 2H, 2×Ar-H), 7.48 (m, 2H, 2×Ar-H), 7.43-7.39 (m, 3H, 3×Ar-H), 4.27 (d, 1H, $J_{1,2}$=8.4 Hz, H-1), 4.10 (bd, 1H, *J*=10.1 Hz, *CH*NSO_2), 3.89 (bt, 1H, H-5), 3.77 (bd, 1H, H-6b), 3.65 (dd, 1H, $J_{2,3}$=9.2 Hz, 1H, H-2), 3.53 (dd, $J_{6a,6b}$=15.8 Hz, $J_{5,6a}$=9.0 Hz, 1H, H-6b), 3.48 (dd, $J_{3,4}$=9.5 Hz, 1H, H-3), 3.41 (s, 3H, OMe), 3.05 (dd, 1H, $J_{4,5}$=9.0 Hz, 1H, H-4), 2.19 (m, 1H, *CH*Me_2), 1.97 (s, 3H, N*H*Ac), 1.11 (d, J_{vic}= 6.4 Hz, 3H, *Me*$_2$CH), 0.98 (d, 3H, J_{vic}=7.2 Hz, *Me*$_2$CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.3 (C=O), 149.4 (Ar-*C*-SO_2), 143.2, 140.2, (2×Ar-*C*), 132.7-130.8 (Ar-*C*H), 106.1 (C-1), 79.3, 78.6, 77.1, (C-3, C-4, C-5), 59.9, 59.6 (C-2, *CH*NSO_2), 48.4 (C-6), 32.3 (*CH*Me_2), 25.4, 22.9 (CH*Me*$_2$), 23.9 (*Me*CO). Elemental Analysis for $C_{26}H_{34}N_2O_9S$ calculated: % C, 56.71; % H, 6.22; % N 5.09; found: % C, 56.79; % H, 6.27; % N 5.08.

4.1.9.4. (2*R*)-3-methyl-2-(*N*-(((2*R*,3*S*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-methoxytetrahydro-2H-pyran-2-yl)methyl)biphenyl-4-ylsulfonamido)butanoic acid (11). The title compound was prepared from the protected carboxylic acid **9** following the general procedure. The trituration with Et_2O afforded pure **11** (98% yield) as a white solid; mp. 95-98 °C; ^1H NMR (250 MHz, CD_3CN): δ 8.01 (m, 2H, 2×Ar-H), 7.89-7.61 (m, 4H, 4×Ar-H), 7.58-7.38 (m, 3H, 3×Ar-H), 4.11 (d, 1H, $J_{1,2}$=7.8 Hz, H-1), 3.97 (bs, 1H, *CH*NSO_2), 3.70 (m, 1H, H-5), 3.58-3.23 (m, 5H, H-3, H-4, H-6b, 2×OH), 3.49 (s, 3H, OMe), 3.19 (m, 1H, H-6a), 3.01 (m, 1H, H-2), 2.13 (m, 2H, *CH*Me_2, OH), 1.14 (bs, 3H, CHMe_2), 0.96 (d, 3H, J_{vic}=6.5 Hz, CHMe_2). ^{13}C NMR (62.9 MHz, CD_3CN): δ 171.3 (C=O), 146.5 (Ar-*C*-SO_2), 140.7, 138.8 (2×Ar-*C*), 130.1-128.3 (Ar-*C*H), 105.4 (C-1), 77.8, 77.1, 75.0, 74.2 (C-2, C-3, C-4, C-5), 66.9 (*CH*NSO_2), 57.8 (OMe), 48.1 (C-6), 28.3 (*CH*Me_2), 21.7, 19.9 (CHMe_2). Elemental Analysis for $C_{24}H_{31}NO_9S$ calculated: % C, 56.57; % H, 6.13; % N 2.75; found: % C, 56.60; % H, 6.18; % N 2.70.

4.1.9.5. (2*R*)-3-methyl-2-(*N*-(2-(3-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)thioureido)ethyl)biphenyl-4-ylsulfonamido)butanoic acid (13). The title compound was prepared from the protected carboxylic acid **12** following the general procedure. The trituration with Et_2O afforded pure **13** (85.5% yield) as a white solid; mp. 165-168 °C; ^1H NMR (400 MHz, CD_3OD): δ 7.97 (m, 2H, 2×Ar-*H*), 7.77 (m, 2H, 2×Ar-*H*), 7.68 (m, 2H, 2×Ar-*H*), 7.47 (m, 2H, 2×Ar-*H*), 7.39 (m, 2H, 2×Ar-*H*), 4.20-3.80 (m, 6H, H-1, H-6b, C*H*NSO_2, CH_2NSO_2, CH_2NHCS), 3.78-3.35 (m, 4 H, H-3, H-4, H-5, H-6a), 3.25 (m, 1H, CH_2NSO_2), 2.12 (m, 1H, *CH*Me_2), 1.00 (d, 3H, J_{vic}=6.4 Hz, CHMe_2), 0.95 (bd, 3H, CHMe_2). ^{13}C NMR (100 MHz, CD_3OD): δ 186.2 (C=S), 175.0 (C=O), 145.3 (Ar-*C*-SO_2), 139.4, 139.3 (2×Ar-*C*), 128.7-126.9 (Ar-*C*H), 83.1 (C-1), 77.6, 77.4, 72.7, 70.1 (C-2, C-3, C-4, C-5), 66.5 (*CH*NSO_2), 61.4. (C-6), 45.0, 43.2 (CH_2NSO_2, CH_2NHCS), 28.4 (*CH*Me_2); 20.7, 18.8 (CHMe_2). Elemental Analysis for $C_{26}H_{35}N_3O_9S_2$ calculated: % C, 52.25; % H, 5.90; % N 7.03; found: % C, 52.31; % H, 5.96; % N 7.00.

4.1.9.6. (2*R*)-3-methyl-2-(*N*-(2-(3-(((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-methoxytetrahydro-2H-pyran-2-yl)methyl)thioureido)ethyl)biphenyl-4-ylsulfonamido)butanoic acid (15). The title compound was prepared from the protected carboxylic acid **14** following the general procedure. The trituration with Et_2O followed by a flash cromathography using an Isolute Flash Si II cartridge (CH_2Cl_2-MeOH in gradient from 20:1 to 6:1) afforded pure **15** (73% yield) as a white foam. ^1H NMR (400 MHz, CD_3OD): δ 7.94 (m, 2H, 2×Ar-H), 7.80 (m, 2H, 2×Ar-H), 7.69 (m, 2H, 2×Ar-H), 7.48 (m, 1H, 2×Ar-H), 7.40 (m, 1H, Ar-*H*), 4.17 (d, $J_{1,2}$=7.8 Hz, 1H, H-1), 4.02 (d, *J*=10.4 Hz, 1H, $CHNSO_2$), 3.95-3.78 (m, 4H, H-6b, CH_2NSO_2, CH_2NCS), 3.51 (s, 3H, OMe), 3.41-3.02 (m, 4H, H-3, H-4, H-5,

CH_2NSO_2), 3.23-3.14 (m, 2H, H-2, H-6a), 2.24 (m, 1H, C*H*Me2), 1.31 (d, J_{vic}=6.5 Hz, 3H, CH*Me2*), 1.01 (d, J_{vic}=6.6 Hz, 3H, CH*Me2*). ^{13}C NMR (100 MHz, CD_3OD): δ 183.2 (C=S), 173.8 (C=O), 147.0 (Ar-*C*-SO_2), 140.6, 138.9 (2×Ar-*C*), 130.1-128.3 (Ar-*C*H), 105.4 (C-1), 77.3, 76.1 (C-4, C-5), 75.1, 72.4 (C-2, C-4), 67.9 (*CH*NSO_2), 57.4 (OMe), 47.1 (C-6), 45.3, 45.2 (CH_2NSO_2, *C*H_2NHCS), 29.8 (*CH*Me_2), 20.7, 19.8 (CH*Me2*). Elemental Analysis for $C_{27}H_{37}N_3O_9S_2$ calculated: % C, 53.01; % H, 6.10; % N 6.87; found: % C, 53.10; % H, 6.17; % N 6.83.

4.2. MMP inhibition assays

Pro-MMP-12 was purchased by R&D Systems and pro-MMP-9 was purchased from Calbiochem (Merck-Millipore). Pro-MMP-9 was activated immediately prior to use with *p*-aminophenylmercuric acetate (1 mM APMA for 1 h at 37 °C). Pro-MMP-12 was autoactivated by incubating in Fluorometric Assay Buffer (FAB: Tris 50 mM, pH 7.5, NaCl 150 mM, $CaCl_2$ 10 mM, Brij-35 0.05%, and DMSO 1%) for 30 h at 37 °C. For assay measurements, each inhibitor stock solution (DMSO, 10 mM) was further diluted in FAB at seven different concentrations. Activated enzyme (final concentrations of 1.3 nM for MMP-9 and 2.3 nM for MMP-12) and inhibitor solutions were incubated in the assay buffer for 3 h at 25 °C. After the addition of 200 µM solution of the fluorogenic substrate Mca-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2 (Bachem) for all the enzymes in DMSO (final concentration of 2 µM for all enzymes), the hydrolysis was monitored every 10 s for 15 min, recording the increase in fluorescence (λ_{ex} = 325 nm, λ_{em} = 400 nm) with a Molecular Devices SpectraMax Gemini XPS plate reader. The assays were performed in triplicate in a total volume of 200 µL per well in 96-well microtiter plates (Corning black, NBS). Control wells lack inhibitor. The MMP inhibition activity was expressed in relative fluorescent units (RFU). Percent of inhibition was calculated from control reactions without the inhibitor. IC_{50} was determined using the formula $v_i/v_0 = 1/(1 + [I]/IC_{50})$, where v_i is the initial velocity of substrate cleavage in the presence of the inhibitor at concentration [I] and v_o is the initial velocity in the absence of the inhibitor. Results were analyzed using SoftMax Pro software (version 5.4.3, Molecular Devices, Sunnyvale, CA) and Prism Software version 5.0 (GraphPad Software, Inc., La Jolla, CA).

4.3. Evaluation of intestinal permeability by using an *ex vivo* everted gut sac model

Male Wistar rats (300-350 g) were taken from ENVIGO (Milan, Italy). All the experimental procedures were performed according to european (EEC Directive 2010/63) and italian (D.L. 4 March 2014 n.26)

legislation. Rats were housed in cages with free access to standard food pellets and water on a 12h light/dark cycle.

Animals were fasted overnight and anesthetized with an intraperitoneal injection of sodium tiopenthal (100 mg/Kg); then, small intestine of each animal was quickly excised and placed in Krebs solution (composition of saline: $NaHCO_3$ 0.025M; Glucose 0.012M; NaCl 0.118M; KCl 0.005M; $MgSO_4 \times 7H_2O$ 0.0012M; $CaCl_2 \times 2H_2O$ 0.0025M; KH_2PO_4 0.0012M, pH 7.4) continuously gassed with Clioxicarb, a mixture of O_2 (95%) and CO_2 (5%). According with Everted Gut Sac model, small intestine was gently everted, filled with Krebs solution at 37 °C and divided into sacs approximately 3 cm length, using suture silk. Sacs were then placed in triplicate in tubes containing 30 mL of Krebs solution, medicated with compound **1**, **2**, **3**, **12**, **13** (100 μM) or with their vehicle (DMSO 0.1%) and gassed with Clioxicarb continuously. Then, the sacs were incubated at 37 °C in an oscillating water bath, and at the selected time points (0, 15, 30 and 60 min), they were removed, washed in Krebs solution and blotted dry. Finally, they were cut open and serosal fluid was drained into small tubes. The remaining external solution was also collected. Serosal fluid and a sample of external solution were analysed by HPLC, in order to quantify the intestinal absorption of tested compounds.

In a second series of experiments, the possible role of two different glucose transporters in the intestinal permeability of the most promising derivative (compound **3**) has been investigated. In particular, phloridzin dihydrate 10 μM and 100 μM (Sigma-Aldrich, a selective inhibitor of sodium-glucose transport protein 1, SGLT1) or the GLUT2 inhibitor phloretin 100 μM (Sigma-Aldrich) were incubated for 10 min before adding compound **3**.

4.3.1. Quantification of intestinal permeability by HPLC

Preparation of calibration standards

Standard stock solutions of compounds **1**, **2**, **3**, **12** and **13**, were prepared by dissolving an accurately weighted amount of compound in DMSO with a final concentration of 10 mM. To build-up the calibration curves for each compound, aliquots from these stock solutions were diluted with Krebs solution to prepare samples with final concentration of 500, 300, 100, 50, 10, 1 μM. The peak area (Y) and the concentrations of compounds (X) were used to plot the calibration curves. The linear correlation coefficients (r^2) for the tested compounds were: 0.998 (compound **1**), 0.984 (compound **2**), 0.999 (compound **3**), 0.995 (compound **12**), and 0.999 (compound **13**).

Chromatographic conditions

HPLC analysis were conducted with a Merck Hitachi liquid chromatography system consisting of a L-6200 Intelligent Pump, a L-4500A Diod Array Detector, a T-6300 column Thermostat and a Jasco DG-980-50 degasser. Separations were performed using a Kromasil Eternity (C18, 2.5 μm) analytical column (4.6 × 100 mm) equipped with a Phenomenex Security guard cartridge (C18, 4 × 3.0 mm ID). The injection volumes were of 20 μL. Chromatographic analysis were performed in gradient mode, with the mobile phase consisting of eluent A (H_2O with 0.01% of TFA) and eluent B (CH_3CN), the flow rate was of 1.5 mL/min and the monitoring wavelength was set at 254 nm. The full equilibration time between two runs was of 10 min to ensure column re-equilibration prior to the following injection.
*Compound **1***. The mobile phase gradient program (A/B) was: 60/40 (t = 0 min), 0/100 (t = 15 min) 0/100 (t = 16 min), 60/40 (t = 18 min). t_R 6.2 min.
Compound **2**. The mobile phase gradient program (A/B) was: 60/40 (t = 0 min), 0/100 (t = 7 min) 0/100 (t = 7.5 min), 60/40 (t = 10 min). t_R 5.0 min.
*Compound **3***. The mobile phase gradient program (A/B) was: 90/10 (t = 0 min), 36/64 (t = 6 min), 0/100 (t = 7 min) 0/100 (t = 7.5 min), 90/10 (t = 9 min). t_R 6.4 min.
*Compound **12***. The mobile phase gradient program (A/B) was: 60/40 (t = 0 min), 30/70 (t = 8 min), 0/100 (t = 9 min), 60/40 (t = 11 min). t_R 5.6 min.
*Compound **13***. The mobile phase gradient program (A/B) was: 90/10 (t = 0 min), 36/64 (t = 9 min), 0/100 (t = 11 min) 0/100 (t = 13 min), 90/10 (t = 16 min). t_R 8.2 min.
Evaluation of the absorption mechanism
For the evaluation of the absorption mechanism of glycoconjugate inhibitor **3**, the quantification of the absorbed compound was determined in presence of phloridzin 10 μM and 100 μM and phloretin 100 μM. The HPLC analyses were performed using the same conditions described above for compound **3**.

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Supplementary Data
Supplementary data associated with this article can be found, in the online version, at

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