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Sugar-Based Arylsulfonamide Carboxylates as Selective and Water-Soluble Matrix Metalloproteinase-12 Inhibitors

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Matrix metalloproteinase-12 (MMP-12) can be considered an attractive target to study selective inhibitors useful in the development of new therapies for lung and cardiovascular diseases. In this study, a new series of arylsulfonamide carboxylates, with increased hydrophilicity resulting from conjugation with a β-*N*-acetyl-D-glucosamine moiety, were designed and synthesized as MMP-12 selective inhibitors. Their inhibitory activity was evaluated on human MMPs by using the fluorimetric assay, and a crystallographic analysis was performed to characterize their binding mode. Among these glycoconjugates, a nanomolar MMP-12 inhibitor with improved water solubility, compound **3** [(*R*)-2-(*N*-(2-(3-(2-acetamido-2-deoxy-β-D-glucopyranosyl)thioureido)ethyl)biphenyl-4-ylsulfonamido)-3-methylbutanoic acid], was identified.

Introduction

MMP-12, or macrophage metalloelastase, belongs to the matrix metalloproteinase (MMP) family of enzymes, a well-known class of endopeptidases able to degrade all the components of the extracellular matrix (ECM). MMP-12 is a zinc-dependent enzyme mainly produced by macrophages, and its principal substrates are elastin, the major constituent of alveolar walls, and other proteins such as type IV collagen, fibronectin, laminin, gelatin, vitronectin, and chondroitin sulfates.[1] MMP-12 was previously shown to be involved in acute and chronic pulmonary inflammatory diseases such as chronic obstructive pulmonary disease (COPD) and emphysema.[2] Moreover, overexpression of MMP-12 was reported to be associated with lung cancer. In particular, the clinical importance of MMP-12 was demonstrated in non-small cell lung cancer (NSCLC) development[3] with a critical role played by MMP-12 upregulation in the transition from emphysema to lung cancer.[4] Finally, MMP-12 was previously shown to be critical in the initiation and progression of atherosclerosis by stimulating the transformation of fatty streaks into fibrous plaques.[5] These observations suggest that MMP-12 selective inhibitors could be potentially useful for the treatment of lung and cardiovascular diseases. Selective MMP-12 inhibitors were recently reported by Dive et al. for atherosclerosis (RXP470.1),[6] by Li et al. for the potential treatment of asthma (MMP145),[7] and by Astra Zeneca for COPD (AZD1236),[8] among others. The principal obstacles that hinder clinical development of MMP inhibitors (MMPIs) are related to inadequate selectivity for the target enzyme, poor water solubility with consequent poor oral bioavailability, and long-term toxicity. Good water solubility of MMPIs is highly desirable above all for inhibitors used in the topical treatment of lung pathologies, such as emphysema and COPD, which require good solubility in aqueous biological fluids. Compounds with improved hydrophilicity are likely to have better bioavailability and fewer side effects owing to cross-inhibition of anti-target zinc proteases (caused by high dosage).[9] Therefore, to obtain potent and selective MMP-12 inhibitors endowed with increased water solubility, we undertook the design and synthesis of a series of arylsulfonamide carboxylates conjugated with a β-*N*-acetyl-D-glucosamine moiety, compounds **2**–**11** (Figure 1). Our starting hypothesis was that the insertion of a sugar moiety in the proper position of the arylsulfonamido scaffold could increase water solubility without interfering with the potency of the inhibitor.[10] To prove that, the inhibitory activities of the new glycoconjugates were tested in vitro by using recombinant MMPs, and X-ray crystallographic studies were performed to rationalize the re-

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Supporting Information (synthesis and characterization of all intermediate compounds and detailed crystallographic data) and the ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10.1002/cmdc.201600235.

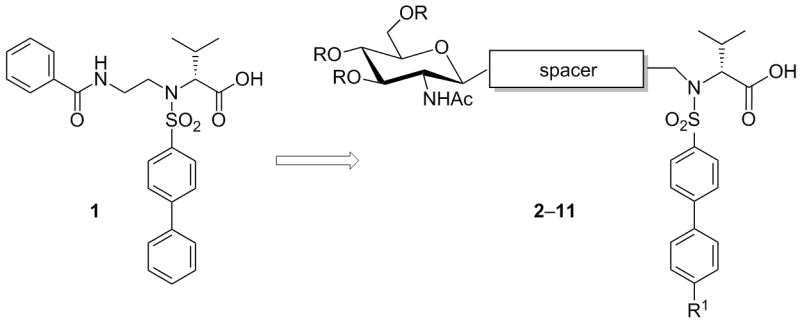


Figure 1. Structure of reference compound **1** and glycoconjugated analogues **2**–**11**.

sults. Furthermore, the physicochemical properties of the most promising derivatives were evaluated.

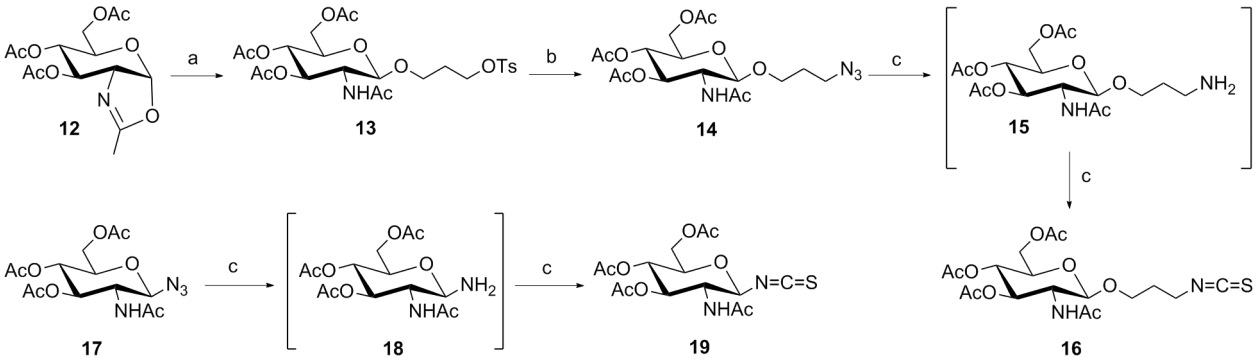
Results and Discussion

Design of glycoconjugate MMP-12 inhibitors

Previous studies by Attolino et al.[11] proved that the introduction of hydrophilic chains on the nitrogen atom of a hydroxamate-based sulfonamide scaffold does not substantially change the affinity of the inhibitors for MMPs. On the basis of these findings, in the present study we modified the structure of recently reported carboxylate MMP-12 inhibitor **1**[12] (Figure 1) by introducing a glycosidic residue on its sulfonamide nitrogen atom (P2′ substituent) through insertion of a suitable spacer. This choice was also supported by the X-ray structure of the **1**–MMP-12 complex,[12] which showed that the benzamidoethyl group does not directly participate in binding. β-*N*-Acetyl-D-glucosamine (GlcNAc) was chosen for the preparation of novel inhibitors **2**–**11** (Figure 1), because it is a very common monosaccharide in nature. In fact, GlcNAc is present in many glycostructures (e.g., chitin and chito-oligomers), is crucial for protein and lipid glycosylation, and has a relevant role in the synthesis of the aberrant glycosylated molecules on cancer cell surfaces (multiantennary structures).[13] The conjugation between GlcNAc and the MMP inhibitor scaffold was achieved through the introduction of a thioureido group (compounds **2**–**5**, **10**, and **11**; Tables 1 and 3) or a 1,2,3-triazole group (compounds **6**–**9**, Table 2). These groups were chosen as linkers because 1,2,3-triazoles show topological and electronic similarities with amide bonds and for their inertness in vivo with respect to oxidation and reduction processes,[14] whereas the thioureido group is biocompatible and stable in most biological systems. A *n*-propyloxy chain was inserted between the inhibitor and the sugar moiety in both series of derivatives, compounds **4**/**5** (Scheme 3) and **8**/**9** (Scheme 4), to evaluate the influence of a longer spacer on the affinity for the enzyme. Finally, sugar-protected analogues, acetylated on the hydroxy groups (compounds **2**/**4** and **6**/**8**), were also evaluated.

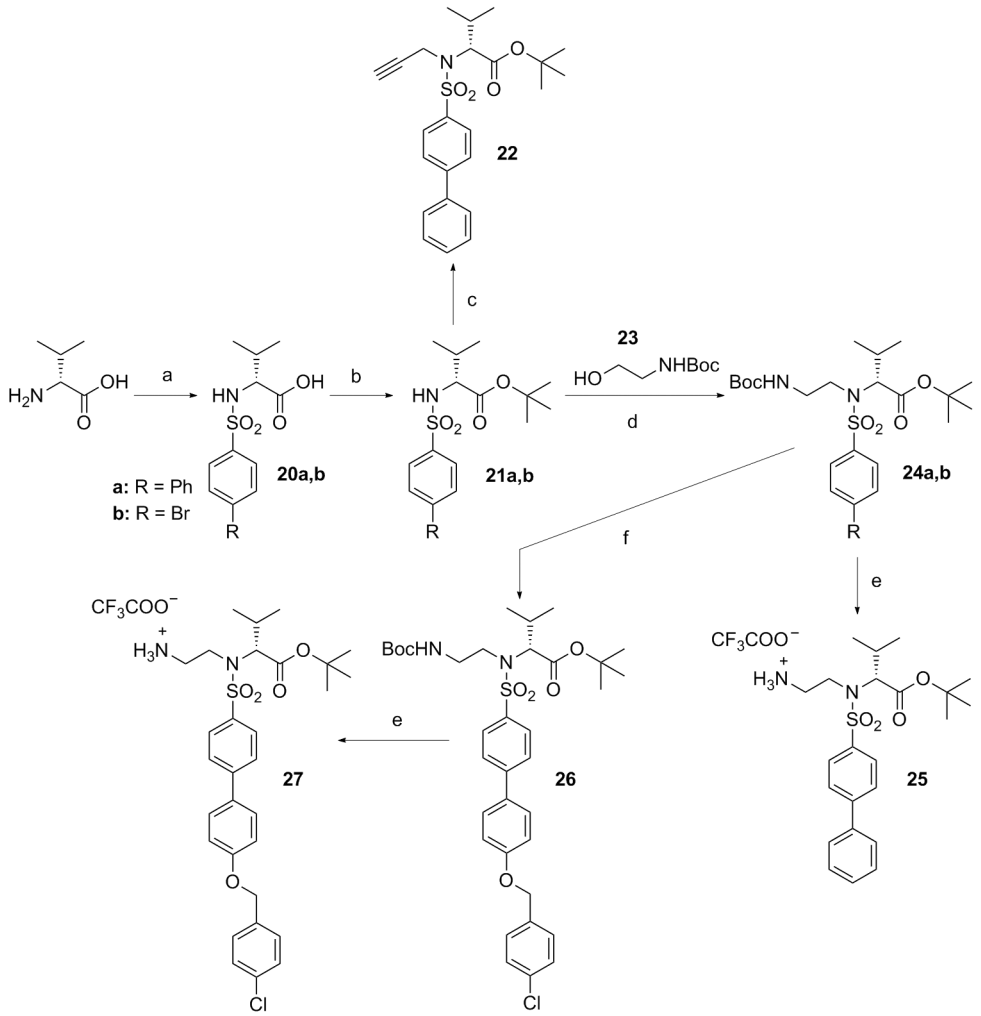
Chemistry

The preparation of all sugar moieties used for the coupling with the MMP inhibitor is shown in Scheme 1. Known oxazoline **12**[15] and azide **17**[16] were obtained in two steps from commercial D-glucosamine hydrochloride according to published procedures. Glycosylation of **12** with 3-*O*-tosyl-1,3-propandiol [$HO(CH_2)_3OTs$][17] was conducted in 1,2-dichloroethane (DCE) with camphorsulfonic acid (CSA) as the catalyst, which afforded β-glycoside **13** ($J_{1,2}$ = 8.5 Hz) stereoselectively in good yield (69%). Known azidopropyl *N*-acetyl-D-glucosamine **14**[18] was prepared by conversion of **13** into the azide through an S_N2 reaction [NaN_3, tetrabutylammonium iodide (TBAI), DMF] in nearly quantitative yield (98%). Azido compounds **14** and **17**[16]



Scheme 1. Synthesis of sugar intermediates **14**, **16**, **17**, and **19**. *Reagents and conditions*: a) CSA, $HO(CH_2)_3OTs$, 4 Å molecular sieves, DCE, 80 °C, 12 h, 69%; b) NaN_3, Bu_4NI, DMF, 50 °C, 1 h, 98%; c) 1. PPh_3-resin, THF/H_2O (20:1), 24 h; 2. DPT, CH_2Cl_2, 1.5–3 h, 46–64% (from azide).

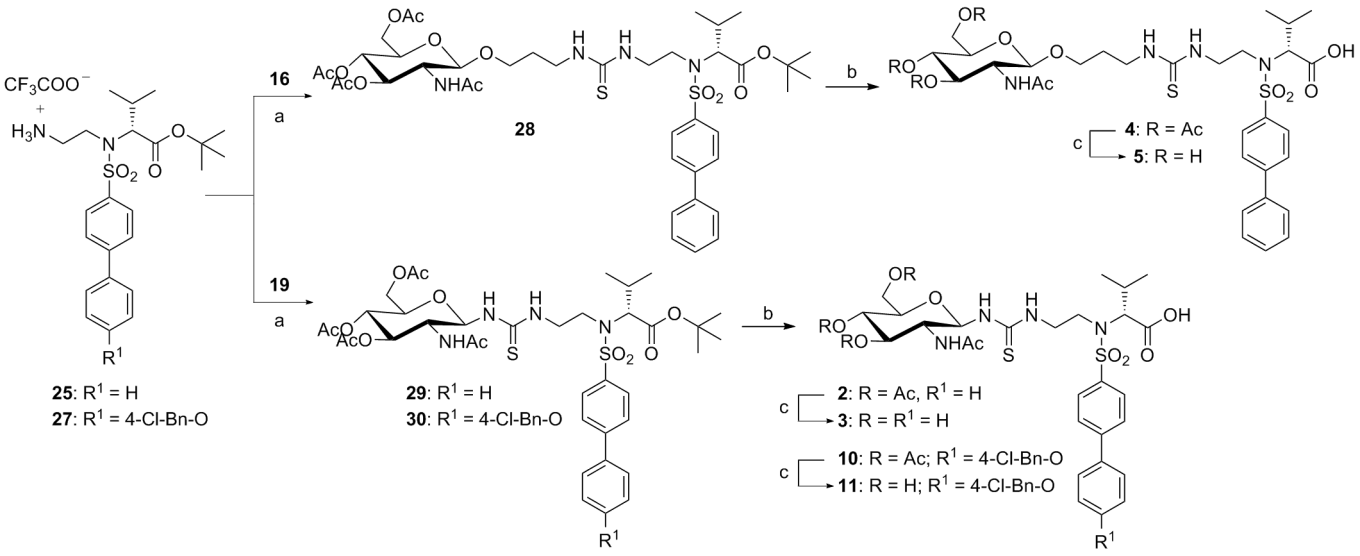
were reduced to corresponding amines **15**[19] and **18**[16] (Scheme 1) in quantitative yields under Staudinger conditions (resin-PPh_3, THF/H_2O). Without further purification, amines **15** and **18** were transformed into corresponding isothiocyanate derivatives **16** and **19** through treatment with di-2-pyridyl thionocarbonate (DPT). DPT[20] is a commercially available, solid, nontoxic reagent that can be used in the preparation of isothiocyanates as a safe alternative to thiophosgene. After 5–24 h, intermediate β-glycosyl isothiocyanates **16** (64% yield from **14**) and **19** (46% yield from **17**) were obtained after removal of the solvents and purification by flash chromatography. MMP inhibitor precursors **22**, **25**, and **27** were prepared as described in Scheme 2. Commercially available biphenyl-4-sulfonyl chloride and *p*-bromobenzenesulfonyl chloride were respectively converted into sulfonamides **20a** and **20b** by reaction with D-valine in H_2O and dioxane in the presence of triethylamine as the base (44–45% yield) according to a previously reported procedure.[21] Sulfonamides **20a** and **20b** were then protected on the carboxylic moiety as *tert*-butyl esters (*R*)-**21a** and (*R*)-**21b** by using *N*,*N*-dimethylformamide-di-*tert*-butyl acetal at 95 °C (46–60% yield). Sulfonamide **21a** was N-alkylated by treatment with propargyl bromide in DMF by using potassium carbonate as the base to afford alkyne **22** in 82% yield. Mitsunobu condensation of sulfonamides **21a** and **21b** with commercial *tert*-butoxycarbonyl (Boc)-protected ethanolamine **23** in the presence of diisopropyl azodicarboxylate (DIAD) and triphenylphosphine gave *tert*-butyl esters (*R*)-**24a** and (*R*)-**24b** (62–85% yield). Pd-catalyzed Suzuki coupling of commercially available 4-(4′-chlorobenzyloxy)phenylboronic acid with *p*-bromo ester **24b** in the presence of K_3PO_4 afforded (*R*)-*tert*-butyl ester **26** in 80.6% yield. Finally, amines **25** and **27** were obtained as trifluoroacetate salts by selective hydrolysis of the Boc group in the presence of the *tert*-butyl group upon treatment of compounds **24a** and **26** with trifluoroacetic acid (TFA) under controlled conditions (2 h at 0 °C under a argon atmosphere, 74–78% yield). Thioureido derivatives **2**–**5**, **10**, and **11** were obtained through coupling of MMP inhibitor amino precursors **25** and **27** with appropriate isothiocyanate β-*N*-



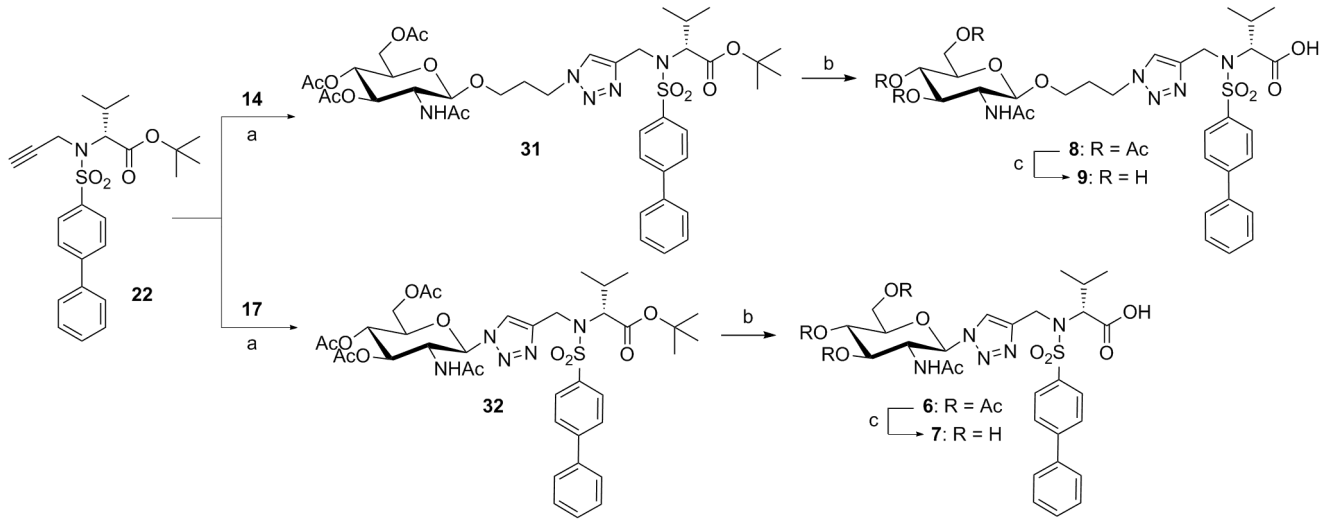
Scheme 2. Synthesis of MMP inhibitor intermediates **22**, **25**, and **27**. *Reagents and conditions*: a) biphenyl-4-sulfonyl chloride or *p*-bromobenzenesulfonyl chloride, Et_3N, H_2O, dioxane, RT, 18 h, 44–45%; b) $(CH_3)_2NCH[OC(CH_3)_3]_2$, toluene, 95 °C, 4 h, 46–60%; c) propargyl bromide, K_2CO_3, DMF, RT, 48 h, 82.6%; d) PPh_3, DIAD, THF, RT, 18 h, 62–85%; e) TFA, CH_2Cl_2, 0 °C, 2 h, 74–78%; f) 4-(4′-chlorobenzyloxy)phenylboronic acid, K_3PO_4, $Pd(PPh_3)_4$, dioxane/H_2O (1:4.4), 70 °C, 2 h, 80.6%.

acetyl-D-glucosamine derivative **16** or **19** (Scheme 3), whereas copper-catalyzed azide–alkyne cycloaddition (CuAAC)[22] was used to connect MMP inhibitor alkynyl precursor **22** to azide β-*N*-acetyl-D-glucosamine derivatives **14** and **17** (Scheme 4) to give 1,2,3-triazole derivatives **6**–**9**. Condensation between isothiocyanates **16** and **19** and MMP inhibitor precursors **25** and **27** (Scheme 3) was performed in CH_2Cl_2/DMF (4:1) in the presence of Et_3N at 60 °C for 12–24 h. Purification of the crude product by flash chromatography yielded pure **29** (96%), whereas thiureido derivatives **28** and **30** were obtained with an impurity of the corresponding isothiocyanates (≈5%, determined by NMR spectroscopy). Their structures were confirmed by the following reactions. β-Glycosyl azides **14** and **17** were conjugated to alkyne **22** (Scheme 4) by CuAAC click chemistry according to a reported procedure.[22] The reactions were performed in a mixture DMF/H_2O (4:1) with a copper(II) sulfate and sodium ascorbate catalytic system and were heated under microwave irradiation at 80 °C for 30 min. Desired 1,2,3-triazole derivatives **31** and **32** were isolated after purification by flash chromatography with complete regiospecificity in good yields (81 and 94%, respectively).

Analysis of **31** and **32** by NMR spectroscopy (^1H, ^{13}C, and 2D NMR experiments) confirmed their structures. The exclusive formation of 1,4-disubstituted 1,2,3-triazoles **31** and **32** was identified by the large Δ (δC_4–δC_5) values (≈20 ppm) observed in the ^{13}C NMR spectra of the cycloadducts. Removal of the *tert*-butyl group in **28**–**32** (Schemes 3 and 4) by treatment with TFA gave desired carboxylic acids **4**, **2**, **10**, **8**, and **6** after trituration of the crude products with Et_2O. Finally, de-*O*-acetylation of **4**, **2**, **10**, **8**, and **6** by treatment with 3.5 N NH_3/MeOH afford-



Scheme 3. Synthesis of thioureido derivatives **2**–**5**, **10**, and **11**. *Reagents and conditions*: a) Et_3N, CH_2Cl_2/DMF (4:1), 60 °C, 20–24 h, 96%; b) CF_3COOH, CH_2Cl_2, RT, 24 h, 92%; c) 7 N NH_3/MeOH, MeOH, RT, 20–24 h, 79–94%.



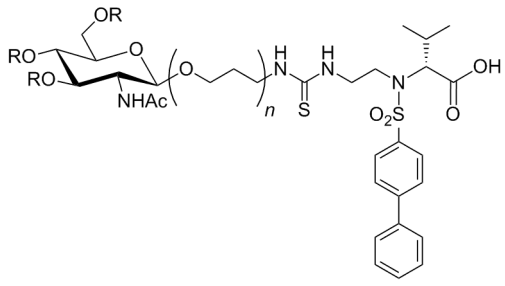
Scheme 4. Synthesis of 1,2,3-triazole carboxylates **6**–**9**. *Reagents and conditions*: a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF/H_2O (4:1), microwave, 80 °C, 30 min, 81–94%; b) CF_3COOH, CH_2Cl_2, RT, 24 h, 55–81%; c) 7 N NH_3/MeOH, MeOH, RT, 20–24 h, 67–70%.

ed deprotected derivatives **5**, **3**, **11**, **9**, and **7** in good yields (67–94%) after trituration of the crude products with Et_2O. The NMR spectra (^1H, ^{13}C, and 2D NMR experiments) and elemental analyses of all the compounds confirmed the structures shown.

MMP inhibition and crystallographic studies

The first series of carboxylic acids synthesized, that is, thioureido derivatives **2**–**5** (Table 1) and 1,2,3-triazole derivatives **6**–**9** (Table 2), were assayed in vitro on human recombinant MMP-12 by using a fluorogenic peptide[23] as the substrate. Inhibitor **1**, devoid of any sugar moiety, was used as the reference compound. Inhibitory activity against MMP-9 (gelatinase B) was chosen at first instance to evaluate selectivity for MMP-12. MMP-9 together with MMP-2 and MMP-8 differ from MMP-12 by having an "intermediate S1′ pocket", whereas the latter is classified as a "deep S1′ pocket MMP".[24] Improved selectivity for MMP-12 over MMP-9 could be indicative of the ability of the new derivatives to discriminate among the various MMP family members. In general, 1) all new derivatives showed stronger affinity for MMP-12 than for MMP-9 and 2) thioureido derivatives **2**–**5** were more active than their triazole analogues, compounds **6**–**9**, on both enzymes. In particular, among the thioureido derivatives the best results were obtained for compound **2**, an *O*-acetylated derivative with a short spacer between the sugar moiety and the biphenyl sulfonamide. The activity of compound **2** on MMP-12 (IC_{50}: 18 nM) was similar to that of reference compound **1** with a fivefold better selectivity over MMP-9. Noteworthy, in both series of compounds the introduction of an *n*-propyloxy chain between the linker group and GlcNAc did not substantially affect activity toward MMP-12. Only in the thioureido series of compounds did acetylation of the hydroxy groups on the carbohydrate moiety lead to a small increase in activity in the compound with a short spacer (i.e., **2** was twofold more active than **3**).

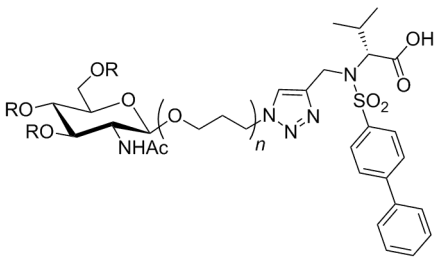
Table 1. MMP-12 and MMP-9 inhibitory activities of thioureido derivatives **2**–**5** and reference compound **1**.



Compd	R	*n*	IC_{50} [nM][a] MMP-12	MMP-9
2	Ac	0	18	1200
3	H	0	40	5400
4	Ac	1	36	1220
5	H	1	28	1200
1			35	510

[a] Values are the average of three determinations with a standard deviation of $<10\%$.

Table 2. MMP-12 and MMP-9 inhibitory activities of 1,2,3-triazole derivatives **6**–**9** and reference compound **1**.



Compd	R	*n*	IC_{50} [nM][a] MMP-12	MMP-9
6	Ac	0	72	4400
7	H	0	53	3100
8	Ac	1	63	6800
9	H	1	73	9400
1			35	510

[a] Values are the average of three determinations with a standard deviation of $<10\%$.

To understand the selectivity of compound **2** for MMP-12 over MMP-9, this inhibitor was co-crystallized with the catalytic domain of both enzymes, and the crystal structure was determined to discern the details of ligand binding (Figure 2).

In MMP-12, the acetylated sugar moiety that in compound **2** replaces the phenyl group of compound **1** improves its positioning to better reflect the change in character from a hydrophobic phenyl group to a hydrophilic sugar (Figure 2d). Differently, in MMP-9 the sugar maintains the same position of the benzene-dicarboxyamide present in compound **1**–**1** (a bifunctional analogue of compound **1**, the X-ray structure of which in complex with the catalytic domain of MMP-9 was previously reported[12]) (Figure 2e). This results in improved MMP-12 inhibition and deterioration of MMP-9 inhibition. The sum of the various effects translates into a substantial increase in the selectivity of **2** for MMP-12 over MMP-9. Comparison of the crystal structures of compounds **2** and **3** with a thioureido linker with those of compounds **6**, **7**, and **8** with a triazole linker in MMP-12 showed that the sugar moieties of the two classes of compounds do not overlap (Figure 3). As noted for compound **2**, the sugar moieties of all sugar derivatives select alternative positions different from that preferred by compound **1** to optimize their binding (Figure 3a).

The absence of the acetyl groups in compound **3** makes the site used by **2** less favorable, as the ligand is forced to dock closer to the MMP-12 surface. The improved activity of the inhibitors with the more flexible thioureido linker is likely to be associated to their ability to select more appropriate positions to improve their binding. On the basis of these findings, to further increase the activity and selectivity for MMP-12 of this class of inhibitors we decided to replace the biphenyl group present in the sulfonamide moiety (P1′ group) of **2** with a 4-(4′-chlorobenzyloxy)biphenyl group. In fact, the introduction of this substituent in P1′ was previously reported to confer good

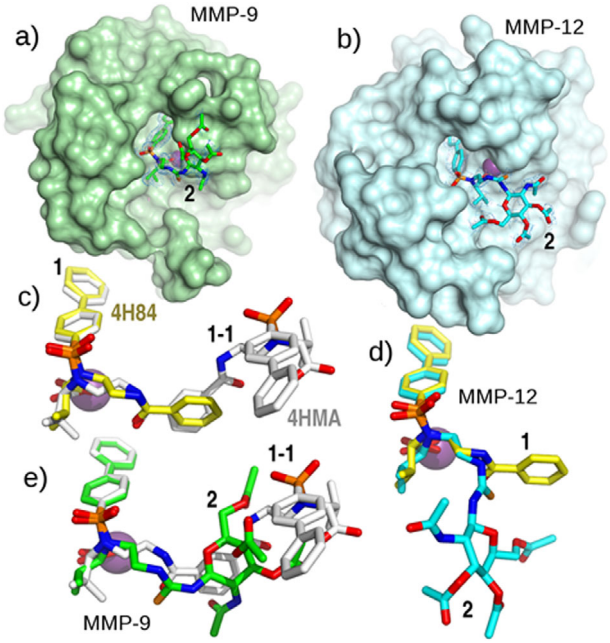
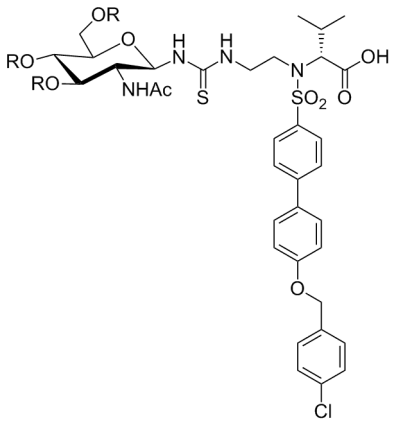


Figure 2. a) Molecular surface of MMP-9 with **2** (PDB ID: 5I12) bound showing the positioning of the sugar moiety. b) Molecular surface of MMP-12 with **2** (PDB ID: 5IOL) in the same orientation. The acetylated sugar moiety fits well in a pocket on the molecular surface. The sugar portion of the compound is in a poor electron density area in both MMP-9 and MMP-12, which indicates that the interactions it makes with either protein are weak and unlikely to contribute to the enthalpy substantially. c) Comparison of the binding of compound **1** to MMP-12 (PDB ID: 4H84)[12] and **1**–**1** bound to MMP-9 (PDB ID: 4HMA)[12] to show they bind in a similar manner to the two different MMPs. d) Comparison of the binding of **1** and **2** to MMP-12 to show the change in position of the acetylated glucosamine of **2** relative to the phenyl ring of **1**. e) Comparison of the binding of compounds **1**–**1** and **2** to MMP-9 to show that the sugar portion follows the same "natural" binding tendency as evidenced by the phenyl group of **1**–**1**.

selectivity for MMP-12 and MMP-13 to similar sulfonamido-based derivatives.[25] 4-(4′-Chlorobenzyloxy)biphenyl thioureido derivatives **10** and **11** (Table 3) were subsequently synthesized and tested on MMP-12 and MMP-9. Unsurprisingly, **10** was the most promising compound, with nanomolar activity against MMP-12 (IC_{50} = 12 nM) and a 70-fold selectivity over MMP-9.

Table 3. MMP-12 and MMP-9 inhibitory activities of 4-(4′-chlorobenzyloxy)biphenyl thioureido derivatives **10** and **11**.



Compd	R	IC_{50} [nM][a]	
		MMP-12	MMP-9
10	Ac	12	860
11	H	42	1470

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

Also for these last compounds, similar to the other thioureido derivatives endowed with a short spacer (compounds **2** and **3**), *O*-acetylation led to a small increase in activity, and sugar-deprotected derivative **11** was less potent (IC_{50} = 42 nM) than its acetylated analogue **10**. At this point, the selectivity profiles of the most promising MMP-12 sugar-based inhibitors, that is, **2**, **3**, and **10**, were evaluated by testing them on MMP-1, MMP-2, and MMP-14 and by comparing the results with those obtained with **1** (Table 4). Compounds **2** and **10** showed similar inhibitory potency on MMP-12 (IC_{50} = 18 and 12 nM, respectively), slightly higher than that of reference compound **1** (IC_{50} = 35 nM). However, as expected, the introduction of the 4-(4′-chlorobenzyloxy)biphenyl group in P1′ led to a great improvement in selectivity over MMP-1 and MMP-14, two MMPs widely believed to be responsible for some side effects that have been clinically observed with the use of broad-spectrum

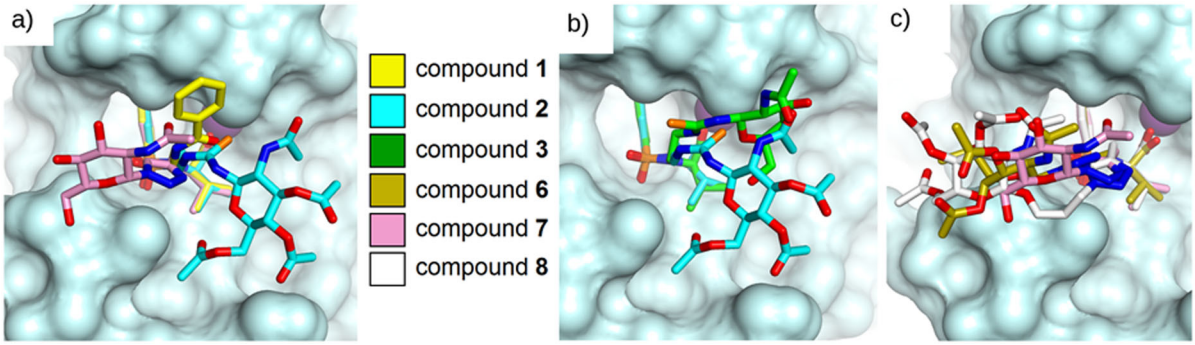


Figure 3. a) Superimpositions of compounds **1** (yellow), **2** (cyan), and **7** (pink) (PDB ID: 5I4O) showing their relative positions in the catalytic domain of MMP-12. b) Compounds **2** and **3** (green) (PDB ID: 5I3M) with the more flexible thioureido linker place their sugar moiety in a totally different orientation relative to those with the triazole linker. c) Compounds **6** (ochre) (PDB ID: 5I2Z) and **7** and **8** (white) (PDB ID: 5I43) with the triazole linker extended along the same path. The only noticeable difference with increasing linker length is a worse definition of the sugar in the electron density, which implies greater mobility.

Table 4. Selectivity profiles of compounds **2**, **3**, and **10** in comparison with that of **1**.

Compd	IC_{50} [nM][a]				
	MMP-1	MMP-2	MMP-9	MMP-12	MMP-14
2	19000	330	1200	18	3900
3	40000	320	5400	40	8900
10	50000	100	860	12	15500
1	16000	170	510	35	1830

[a] Values are the average of three determinations with a standard deviation of $<10\%$.

MMP inhibitors, such as musculoskeletal syndrome (MSS).[26] In fact, 4-(4′-chlorobenzyloxy)biphenyl thioureido derivative **10** displayed a 6-fold increase in selectivity for MMP-12 over MMP-14 and a 41-fold increase in selectivity for MMP-12 over MMP-1 with respect to the selectivity of biphenyl analogue **2** for MMP-12. Compound **3** showed the same potency as **1** on MMP-12 and a selectivity profile similar to that of its sugar-acetylated analogue **2**. In particular, it had 200-fold selectivity for MMP-12 over MMP-14 and 1000-fold selectivity for MMP-12 over MMP-1.

The improvement in hydrophilicity caused by the presence of the glycosidic residue was evaluated by the octanol/water partition coefficient (log *P*). The log *P* values for most active thioureido derivatives **2**, **3**, **10**, and **11** were calculated as clog *P* values, and the results were compared with the value calculated for starting compound **1**, devoid of the glycosidic residue (Table 5). Deprotected glycosidic derivative **3** had a clog *P* value of 3.15, which was lower than that of its acetylated analogue **2** and that of reference compound **1**, with clog *P* values of 4.75 and 5.68, respectively. Also, the water solubility of the new compounds was determined at pH 7.4 (see Table 5), and both glycoconjugates **2** and **3** showed increased solubility (>5 mM) with respect to that of reference compound **1**. Regarding 4-(4′-chlorobenzyloxy)biphenyl thioureido derivatives **10** and **11**, they showed high clog *P* values (>5) and low solubility (≈ 200 μM). In this case, the introduction of the 4-(4′-chlorobenzyloxy)biphenyl portion strongly contributed to improving the lipophilicity of the molecules, which countered the positive effect resulting from the presence of the glycosidic moiety.

Table 5. Physicochemical properties of compounds **2**, **3**, **10**, and **11** in comparison with those of **1**.

Compd	clog *P*[a]	Solubility[b] [μM]
1	5.68	790
2	4.75	>5000
3	3.15	>5000
10	6.81	180
11	5.21	230

[a] Hydrophobicity was calculated as the partition coefficient between octanol and water, log *P* (o/w) by using ACD laboratory software version 14.0 (Advanced Chemistry Development, Inc. Toronto, Canada). [b] Solubility was determined in 50 mM phosphate buffer, pH 7.4, at room temperature after 24 h of equilibration. Concentrations were assessed for supernatants of centrifuged samples by UV/Vis spectroscopy at $\lambda = 270$ nm.

Overall these biological and physicochemical data show that the introduction of a glycosidic portion in P2′, linked through a flexible linker (as in compound **3**), represents a good compromise to maintain nanomolar activity against MMP-12 while boosting bioavailability.

Conclusions

In the present study, a new series of carboxylate-based MMP-12 inhibitors were designed and synthesized starting from previously reported compound **1**. To improve the hydrophilicity and bioavailability of these arylsulfonamides without decreasing their affinity for the target, we linked a β-*N*-acetyl-D-glucosamine (GlcNAc) moiety in P2′. Conjugation between GlcNAc and the MMP inhibitor scaffold was achieved through the introduction of a thioureido group or a 1,2,3-triazole group as the linker. The new glycoconjugates were tested on human MMPs by using a fluorimetric assay. All new derivatives showed stronger affinity for MMP-12 than for MMP-9, and the thioureido derivatives were more active than their triazole analogues. In particular, compound **2**, an *O*-acetylated thioureido biphenyl sulfonamide, was selected for further studies given its high affinity for MMP-12 ($IC_{50} = 18$ nM). Crystallographic analysis provided insight into the binding mode of **2** to MMP-12, its selectivity over MMP-9, and the higher affinity for MMP-12 with respect to that of the triazole analogues. To further optimize its activity and selectivity, the structure of **2** was modified by introducing a 4-(4′-chlorobenzyloxy)biphenyl group in P1′. As expected, 4-(4′-chlorobenzyloxy)biphenyl thioureido derivative **10** showed nanomolar activity against MMP-12 ($IC_{50} = 12$ nM) and 70-fold selectivity over MMP-9. At that point, the physicochemical properties of the most promising derivatives, that is, **2**, **10**, and their respective sugar-deprotected derivatives **3** and **11**, were evaluated, and the results were compared with those obtained with starting compound **1**. Compound **3** was the one able to conjugate a nanomolar activity for MMP-12 and good selectivity over MMPs with improved hydrophilicity (water solubility >5 mM and clog $P = 3.15$). These preliminary results suggest that the introduction of a sugar moiety in P2′, linked through a flexible linker as in compound **3**, not only improves the hydrophilicity of MMP-12 inhibitors but also influences their biological activity on isolated enzymes (higher selectivity over other MMPs). Of course, to fully appreciate the effect of the improved bioavailability of **3** with respect to that of **1**, an in vivo assay will be necessary.

Experimental Section

Chemistry

Melting points were determined with a Kofler hot-stage apparatus. Optical rotations were measured with a PerkinElmer 241 polarimeter at $(20 \pm 2)^\circ$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 above spectrometers operating at

62.9 or 100.57 MHz. The assignments were made, if possible, with the aid of DEPT, COSY, and HSQC experiments. The first-order proton chemical shifts, δ, were referenced to residual solvents. Where indicated, the elemental compositions of the compounds agreed to within 0.4% of the calculated values. 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) was used for column and flash chromatography (70–230 and 230–400 mesh, respectively). Some flash chromatography operations were conducted by the automated system Isolera Four SV (Biotage), equipped with a UV detector with variable wavelength (λ = 200–400 nm) or by 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 were used without further purification. All reactions involving air- or moisture-sensitive reagents were performed under an argon atmosphere by using anhydrous solvents. Anhydrous dimethylformamide (DMF), dichloromethane (CH_2Cl_2), 1,2-dichloethane (DCE), and THF were purchased from Sigma–Aldrich. Other dried solvents were obtained by distillation according to standard procedures[27] and were stored over 4 Å molecular sieves activated for at least 24 h at 200 °C. $MgSO_4$ or Na_2SO_4 was used as the drying agent for solutions. 2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2oxazoline (**12**),[15] 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**17**),[16] and 3-*O*-tosyl-1,3-propandiol[17] were prepared according to reported procedures.

Synthesis

***tert*-Butyl ester 29**: Isothiocyanate **19** (0.49 mmol, 1.1 equiv) was solubilized in dry CH_2Cl_2/DMF (4:1, 26 mL), and a solution of amine salt **25** (0.45 mmol, 1 equiv) in dry CH_2Cl_2/DMF (4:1, 26 mL) containing Et_3N (62 μL, 0.45 mmol) was added dropwise. The mixture was stirred at 60 °C until TLC analysis (EtOAc) revealed the complete disappearance of amine salt **25** (7 h), and then the mixture was concentrated under reduced pressure. Purification by flash chromatography (petroleum ether/EtOAc 2:1) using a Isolute Flash Si II cartridge afforded pure **29** (353 mg, 96% yield calculated from **25**) as a clear syrup; R_f = 0.65 (EtOAc); $[\alpha]_D^{23} = +14.0$ (c = 1.07 in $CHCl_3$); ^{1}H NMR (250.12 MHz, CD_3CN): δ = 7.93–7.78 (m, 4H; Ar-*H*), 7.65 (m, 2H; Ar-*H*), 7.53–7.40 (m, 3H; Ar-*H*), 7.30, 7.16 (2 br s, each 1H; 2N*H*CS), 6.70 (d, $J_{2,NH}$ = 8.7 Hz, 1H; N*H*Ac), 5.25–5.15 (m, 2H; H-1, H-3), 4.97 (dd, $J_{3,4}$ = 9.6 Hz, $J_{4,5}$ = 9.7 Hz, 1H; H-4), 4.23 (dd, $J_{5,6b}$ = 4.4 Hz, $J_{6a,6b}$ = 12.1 Hz, 1H; H-6b), 4.05 (m, 2H; H-6a, H-2), 3.88–3.20 (m, 5H; H-5, C*H*NSO_2, CH_2NSO_2, CH_2NHCS), 3.35 (m, 1H; CH_2NSO_2), 2.25 (m, 1H; Me_2CH), 1.98, 1.97, 1.96 (3 s, each 3H; 3*Me*COO), 1.87 (s, 3H; *Me*CON), 1.18 (s, 9H; Me_3C), 1.00 (d, J_{vic} = 6.3 Hz, 3H; Me_2CH), 0.91 ppm (d, J_{vic} = 6.5 Hz, 3H; Me_2CH); ^{13}C NMR (62.9 MHz, CD_3CN): δ = 184.1 (C=S), 172.7, 171.3, 171.2, 170.6, 170.4 (5C=O), 146.4 (Ar-*C*-SO_2), 139.9, 138.9 (2Ar-*C*), 130–128.2 (Ar-*C*H), 83.8 (C-1), 82.8 (Me_3CO), 73.4 (C-3), 73.2 (C-5), 69.5 (C-4), 67.7 (*C*HN), 62.8 (C-6), 53.6 (C-2), 45.9 (*C*H_2NH), 44.3 (*C*H_2NSO_2), 29.8 (*C*HMe_2), 27.9 (Me_3C), 23.0 (*Me*CON), 21.1, 20.9, 20.8 (3*Me*COO), 20.5, 19.5 ppm (Me_2CH); elemental analysis calcd (%) for $C_{38}H_{52}N_4O_{12}S_2$ (820.97): C 55.59, H 6.38, N 6.82; found: C 55.56, H 6.35, N 6.79.

General procedure for the synthesis of triazole-linked *tert*-butyl esters 31 and **32**: Alkyne **22** (0.1 mmol), sugar azide derivative **14** or **17** (0.11 mmol, 1.1 equiv), $CuSO_4$·5H_2O (0.1 mmol, 1.5 equiv), and sodium ascorbate (0.3 mmol, 3 equiv) were dissolved in DMF/H_2O (4:1, 3.2 mL). The solution was heated under microwave irradiation to 80 °C for 30 min and then diluted with Et_2O (12 mL) and washed with saturated aq $NaHCO_3$ (12 mL); the organic phase was separated, and the aqueous layer was extracted with Et_2O (2× 12 mL). The collected organic extracts were dried ($MgSO_4$·H_2O), filtered, and concentrated under reduced pressure. Purification by flash chromatography (silica gel) gave the pure triazole-linked derivative.

Triazole-linked *tert*-butyl ester 31: Flash chromatography (silica gel, *n*-hexane/EtOAc 3:7 + 0.01% Et_3N) afforded pure **31** (81% yield calculated from **22**) as a clear syrup; R_f = 0.64 (EtOAc); $[\alpha]_D^{23} = +46.2$ (c = 1.06 in $CHCl_3$); ^{1}H NMR (250.12 MHz, CD_3CN): δ = 7.92–7.76 (m, 5H; 4Ar-*H*, C*H*-triazole), 7.69–7.64 (m, 2H; 2Ar-*H*), 7.54–7.40 (m, 3H; 3Ar-*H*), 6.63 (d, $J_{2,NH}$ = 9.2 Hz, 1H; N*H*Ac), 5.16 (dd, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 9.5 Hz, 1H; H-3), 4.95 (dd, $J_{4,5}$ = 9.9 Hz, 1H; H-4), 4.87, 4.69 (AB system, $J_{A,B}$ = 16.6 Hz, 2H; CH_2NSO_2), 4.61 (d, $J_{1,2}$ = 8.5 Hz, 1H; H-1), 4.37 (t, J_{vic} = 7.2 Hz, 2H; CH_2N), 4.22 (dd, $J_{5,6b}$ = 4.9 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H; H-6b), 4.06 (dd, $J_{5,6a}$ = 2.2 Hz, 1H; H-6a), 3.93 (d, J_{vic} = 10.6 Hz, 1H; C*H*NSO_2), 3.85 (ddd, 1H; H-2), 3.72, 3.40 (2 dt, J_{vic} = 6.0 Hz, J_{gem} = 10.6 Hz, each 1H; CH_2O), 3.70 (m, 1H; H-5), 2.22 (m, 1H; Me_2CH), 2.05 (m, 2H; CH_2), 2.00, 1.96, 1.94 (3 s, each 3H; 3*Me*COO), 1.86 (s, 3H; *Me*CON), 1.23 (s, 9H; CMe_3), 0.90 (d, J_{vic} = 6.6 Hz, 3H; Me_2CH), 0.72 ppm (d, J_{vic} = 6.5 Hz, 3H; Me_2CH); ^{13}C NMR (62.9 MHz, CD_3CN): δ = 171.3, 171.1, 171.0, 170.5, 170.5 (5C=O), 146.3, 146.0 (Ar-*C*-SO_2, *C*-triazole), 139.9, 139.8, (2Ar-*C*), 130.1–128.2 (Ar-*C*H), 125.4 (*C*H-triazole), 101.7 (C-1), 82.9 (Me_3CO), 73.5 (C-3), 72.4 (C-5), 69.7 (C-4), 67.7 (*C*HN), 66.9 (CH_2O), 62.9 (C-6), 54.8 (C-2), 47.5 (CH_2N), 41.0 (*C*H_2NSO_2), 30.9 (CH_2), 29.7 (*C*HMe_2), 27.9 (Me_3C), 23.2 (*Me*CON), 20.9 (3*Me*COO), 20.0, 19.4 ppm (Me_2CH); elemental analysis calcd (%) for $C_{41}H_{55}N_5O_{13}S$: C 57.40, H 6.46, N 8.16; found: C 57.37, H 6.41, N 8.25.

Triazole-linked *tert*-butyl ester 32: Flash chromatography on silica gel (*n*-hexane/EtOAc 3:7 + 0.01% Et_3N) afforded pure **32** (94% yield calculated from **22**) as a white foam; R_f = 0.53 (EtOAc); $[\alpha]_D^{23} = +22.0$ (c = 0.9 in $CHCl_3$); ^{1}H NMR (250.12 MHz, CD_3CN): δ = 8.05 (s, 1H; C*H*-triazole), 7.90–7.77 (m, 4H; Ar-*H*), 7.69–7.64 (m, 2H; Ar-*H*), 7.54–7.41 (m, 3H; Ar-*H*), 6.56 (d, $J_{2,NH}$ = 9.2 Hz, 1H; NHAc), 5.19 (d, $J_{1,2}$ = 10.0 Hz, 1H; H-1), 5.43 (dd, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 9.5 Hz, 1H; H-3), 5.17 (dd, $J_{4,5}$ = 9.7 Hz, 1H; H-4), 4.56 (m, 1H; H-2), 4.89, 4.68 (AB system, J_{AB} = 16.7 Hz, 2H; CH_2N), 4.21 (dd, $J_{5,6b}$ = 5.4 Hz, $J_{6a,6b}$ = 12.7 Hz, 1H; H-6b), 4.13–4.04 (m, 2H; H-6a, H-5), 3.88 (d, J_{vic} = 10.5 Hz, 1H; C*H*N), 2,21 (m, 1H; C*H*Me_2), 2.01 (s, 3H; *Me*COO), 1.98 (s, 6H; 2*Me*COO), 1.63 (s, 3H; *Me*COON), 1.20 (s, 9H; Me_3C), 0.89 (d, J_{vic} = 6.6 Hz, 3H; Me_2CH), 0,67 ppm (d, J_{vic} = 6.5 Hz, 3H; Me_2CH); ^{13}C NMR (62.9 MHz, CD_3CN): δ = 171.2, 170.9, 170.8, 170.5, 170.4 (5C=O), 146.3 (Ar-*C*-SO_2, *C*-triazole), 140.0, 139.7 (2Ar-*C*), 130.1–128.2 (Ar-*C*H), 124.8 (*C*H-triazole), 86.1 (C-1), 82.9 (Me_3CO), 75.4 (C-5), 73.2 (C-3), 69.1 (C-4), 67.6 (*C*HN), 62.7 (C-6), 53.9 (C-2), 40.7 (*C*H_2NSO_2), 29.7 (*C*HMe_2), 27.8 (Me_3C), 22.8 (*Me*CON), 20.8 (3*Me*COO), 20.0, 19.4 ppm (Me_2CH); elemental analysis calcd (%) for $C_{38}H_{49}N_5O_{12}S$ (799.89): C 57.06, H 6.17, N 8.76; found: C 57.02, H 6.14, N 8.74.

General procedure for the transformation of *tert*-butyl esters 29, 31, and 32 into carboxylic acids 2, 8, and 6: Ester **29**, **31**, or **32** (0.1 mmol) was dissolved in CH_2Cl_2 (1.7 mL) and treated with CF_3COOH (0.6 mL), this mixture was stirred at 0 °C for 1 h and then at RT. After 24 h, TLC analysis (EtOAc) revealed complete disappearance of the starting material and the formation of a more retained product. The solution was co-evaporated with toluene (4×10 mL) under reduced pressure, and trituration of the crude product with Et_2O afforded the pure carboxylic acid.

Carboxylic acid 2: Yield: 92%, white solid; R_f = 0.41 (EtOAc); mp: 134–135 °C; $[\alpha]_D^{23} = +9.46$ (c = 1.3 in $CHCl_3$); ^{1}H NMR (250.12 MHz,

CD_3OD/D_2O): $\delta = 7.93–7.90$ (m, 2H; Ar-*H*), 7.76–7.73 (m, 2H; Ar-*H*), 7.64 (m, 2H; Ar-*H*), 7.50–7.35 (m, 3H; Ar-*H*), 5.52 (brs, 1H; H-1), 5.19 (dd, $J_{3,4} = 10.3$ Hz, $J_{2,3} = 9.6$ Hz, 1H; H-3), 5.02 (dd, $J_{4,5} = 9.9$ Hz, 1H; H-4), 4.28 (dd, $J_{5,6b} = 3.8$ Hz, $J_{6a,6b} = 12.5$ Hz, 1H; H-6b), 4.20–3.75 [m, 7H; H-6a, H-2, H-5, *C*HNSO$_2$, *C*H$_2$NSO$_2$ (1H), *C*H$_2$NHCS], 3.41 (m, 1H; *C*H$_2$NSO$_2$), 2.25 (m, 1H; Me$_2$*C*H), 1.95 (s, 9H; 3*Me*COO), 1.91 (s, 3H; *Me*CON), 0.98 ppm (m, 2H; *Me*$_2$CH); ^{13}C NMR (62.9 MHz, CD_3OD/D_2O): $\delta = 182.1$ (C=S), 171.7–169.2 (5C=O), 145.8 (Ar-*C*-SO$_2$), 138.9 (2Ar-*C*), 128.9–127.2 (Ar-*C*H), 83.4 (C-1), 72.8, 72.7 (C-3, C-5), 67.7, 67.6 (C-4, *C*HN), 61.8 (C-6), 53.8 (C-2), 43.9, 43.8 (*C*H$_2$NH, *C*H$_2$NSO$_2$), 29.6 (*C*HMe$_2$), 23.1 (*Me*CON), 20.7–20.6 (3*Me*COO), 20.5, 19.6 ppm (*Me*$_2$CH); elemental analysis calcd (%) for $C_{34}H_{44}N_4O_{12}S_2$ (764.86): C 53.39, H 5.80, N 7.33; found: C 53.38, H 5.79, N 7.30.

Carboxylic acid 8: Yield: 55%, white solid; $R_f = 0.24$ ($CHCl_3$/MeOH 95:5); mp: 114–115 °C; $[\alpha]_D^{23} = +6.93$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250.12 MHz, $CD_3CN/CDCl_3$): $\delta = 7.79–7.42$ (m, 11H, *CH*-triazole, 9Ar-*H*, COO*H*), 6.82 (d, $J_{2,NH} = 7.8$ Hz, 1H; NHAc), 5.19 (dd, $J_{2,3} = 10.1$ Hz, $J_{3,4} = 9.6$ Hz, 1H; H-3), 4.94 (t, $J_{4,5} = 9.6$ Hz, 1H; H-4), 4.80–4.45 (m, 2H; *C*H$_2$NSO$_2$), 4.60 (d, $J_{1,2} = 8.5$ Hz, 1H; H-1), 4.30–4.12 (m, 3H; H-6b, *C*H$_2$N), 4.04 (dd, $J_{5,6a} = 1.9$ Hz, $J_{6a,6b} = 12.1$ Hz, 1H; H-6a), 3.87–3.72 (m, 3H; H-5, H-2, *C*HNSO$_2$), 3.70, 3.28 (2brs, each 1H; *C*H$_2$O), 2.28–2.08 (m, 3H; *C*H$_2$, Me$_2$*C*H), 1.98, 1.96, 1.93 (3s, each 3H; 3*Me*COO), 1.85 (s, 3H; *Me*CON), 0.92, 0.83 ppm (2brs, each 3H; *Me*$_2$CH); ^{13}C NMR (62.9 MHz, $CD_3CN/CDCl_3$): $\delta = 171.9$, 171.3, 171.2, 171.0, 170.5 (5C=O), 145.7, 147.6 (Ar-*C*-SO$_2$, *C*-triazole), 139.8, 139.7 (2Ar-*C*), 129.9–128.0 (Ar-*C*H), 128.4 (*C*H-triazole), 101.4 (C-1), 73.2 (C-3), 72.3 (C-5), 69.6 (C-4), 66.5 (*C*H$_2$O), 66.2 (*C*HN), 62.8 (C-6), 54.9 (C-2), 47.5 (*C*H$_2$N), 41.2 (*C*H$_2$NSO$_2$), 30.8 (*C*H$_2$), 30.7 (*C*HMe$_2$), 23.1 (*Me*CON), 20.9 (3*Me*COO), 20.8, 19.8 ppm (*Me*$_2$CH); elemental analysis calcd (%) for $C_{37}H_{47}N_5O_{13}S$ (801.86): C 55.42, H 5.91, N 8.73; found: C 55.40, H 5.89, N 8.70.

Carboxylic acid 6: Yield: 81%, white solid; $R_f = 0.57$ (EtOAc); mp: 144–146 °C; $[\alpha]_D^{23} = +18.7$ ($c = 0.98$ in MeOH); ^{1}H NMR (250.12 MHz, $CDCl_3$): $\delta = 8.06–7.80$ (m, 3H; 2Ar-*H*, *CH*-triazole), 7.74–7.35 (m, 8H; 7Ar-*H*, N*H*), 5.93 (d, $J_{1,2} = 8.9$ Hz, 1H; H-1), 5.31 (dd, $J_{2,3} = 9.3$ Hz, $J_{3,4} = 10.1$ Hz, 1H; H-3), 5.06 (brt, $J_{4,5} = 10.1$ Hz, 1H; H-4),4.81, 4.45 (AB system, $J_{A,B} = 17.1$ Hz, 2H; *C*H$_2$N), 4.65 (m, 1H; H-2), 4.24 (d, $J_{vic} = 9.2$ Hz, 1H; NC*H*), 4.10–3.90 (m, 3H; H-5, H-6a, H-6b), 2.25 (m, 1H; Me$_2$*C*H), 2.04, 2.00, 1.99 (3s, each 3H; 3*Me*COO), 1.84 (s, 3H; *Me*COON), 0.88 (d, $J_{vic} = 4.9$ Hz, 3H; *Me*$_2$CH), 0.68 ppm (d, $J_{vic} = 5.5$ Hz, 3H; *Me*$_2$CH); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 174.6$ (*C*OOH), 171.8, 171.4, 171.3, 169.8 (4C=O), 146.6 (Ar-*C*-SO$_2$, *C*-triazole), 139.7, 139.3 (2Ar-*C*), 129.9–126.0 (Ar-*C*H), 122.0 (*C*H-triazole), 86.8 (C-1), 75.5 (C-5), 73.2 (C-3), 68.8 (C-4), 64.6 (*C*HN), 62.4 (C-6), 53.7 (C-2), 40.4 (*C*H$_2$NSO$_2$), 26.4 (*C*HMe$_2$), 23.6 (*Me*CON), 21.3–21.2 (3*Me*COO), 22.1, 19.4 ppm (*Me*$_2$CH); elemental analysis calcd (%) for $C_{34}H_{41}N_5O_{12}S$ (743.78): C 54.90, H 5.56, N 9.42; found: C 54.88, H 5.54, N 9.39.

Carboxylic acid 4: Isothiocyanate **16** (76 mg, 0.17 mmol) was solubilized in dry CH_2Cl_2/DMF (4:1, 17.5 mL), and a solution of amine **25** (93 mg, 0.17 mmol) in dry CH_2Cl_2/DMF (4:1, 17.5 mL) containing Et$_3$N (50 μL, 2 equiv) was added dropwise. The mixture was stirred at 60 °C for 24 h, until TLC analysis (EtOAc) indicated the formation of a major product ($R_f = 0.42$). The mixture was concentrated under reduced pressure, and the crude residue was taken up in Et$_2$O (10 mL) and washed with H$_2$O (10 mL). The aqueous layer was extracted with Et$_2$O (2×10 mL). The collected organic extracts were combined and dried (MgSO$_4$·H$_2$O), filtered, and concentrated under reduced pressure. The crude residue (136 mg) was submitted to flash chromatography (silica gel, *n*-hexane/EtOAc 2:8) to give a foam solid (125 mg) constituted (NMR) by ester **28** impure of isothiocyanate **16** (5%). Data for **28**: ^{1}H NMR (250.12 MHz, CD_3CN): $\delta = 7.95–7.89$ (m, 3H; 2Ar-*H*, N*H*CS), 7.83–7.77 (m, 2H; 2Ar-*H*), 7.68–7.63 (m, 2H; 2Ar H), 7.53–7.40 (m, 3H; 3Ar-H), 6.89 (d, $J_{2,NH} = 9.4$ Hz, 1H; N*H*Ac), 6.68 (brt, $J = 5.1$ Hz, 1H; N*H*CS), 5.14 (dd, $J_{2,3} = 10.7$ Hz, $J_{3,4} = 9.4$ Hz, 1H; H-3), 4.95 (dd, $J_{4,5} = 9.9$ Hz, 1H; H-4), 4.53 (d, $J_{1,2} = 8.4$ Hz, 1H; H-1), 4.22 (dd, $J_{5,6b} = 4.8$ Hz, $J_{6a,6b} = 12.3$ Hz, 1H; H-6b), 4.05 (dd, $J_{5,6a} = 2.6$ Hz, 1H; H-6a), 3.87 (d, $J_{vic} = 10.4$ Hz, 1H; *C*HNSO$_2$), 4.10–3.45 [m, 6H; H-2, H-5, *C*H$_2$NH (1H), *C*H$_2$NH (1H), *C*H$_2$O (1H), *C*H$_2$NSO$_2$ (1H)], 3.65–3.45 [m, 3H; *C*H$_2$NH (1H), *C*H$_2$O (1H), *C*H$_2$NSO$_2$ (1H)], 3.38–3.25 [m, 1H, *C*H$_2$NH (1H)], 2.21 (m, 1H; Me$_2$*C*H), 1.75 (m, 2H; *C*H$_2$), 2.00, 1.96, 1.95 (3s, each 3H; 3*Me*COO), 1.90 (s, 3H; *Me*CON), 1.19 (s, 9H; *CMe*$_3$), 1.03 (d, $J_{vic} = 6.5$ Hz, 3H; *Me*$_2$CH), 0.92 ppm (d, $J_{vic} = 6.6$ Hz, 3H; *Me*$_2$CH); ^{13}C NMR (62.9 MHz, CD_3CN): $\delta = 183.4$ (C=S), 172.4, 171.3, 171.1, 170.5, 170.3 (5C=O), 146.2 (Ar-*C*-SO$_2$), 139.9, 139.1, (2Ar-*C*), 130.1–128.1 (Ar-*C*H), 101.8 (C-1), 82.7 (Me$_3$*C*O), 73.4 (C-3), 72.3 (C-5), 69.7 (C-4), 69.7 (*C*H$_2$O), 67.6 (*C*HNSO$_2$), 62.9 (C-6), 55.3 (C-2), 45.3, 44.9, 44.8 (2*C*H$_2$N, *C*H$_2$NSO$_2$), 29.4 (*C*H$_2$), 29.8 (*C*HMe$_2$), 27.9 (*Me*$_3$C), 23.3 (*Me*CON), 20.9 (3*Me*COO), 20.5, 19.5 ppm (*Me*$_2$CH). Crude **28** (125 mg) was dissolved in CH_2Cl_2 (2.3 mL), treated with CF_3COOH (0.7 mL), and stirred at 0 °C for 1 h and then at RT. After 24 h, TLC analysis (EtOAc) revealed the complete disappearance of the starting material and the formation of a more retained product ($R_f = 0.26$). The solution was co-evaporated with toluene (4×10 mL) under reduced pressure, and the trituration of the crude product with Et$_2$O afforded pure carboxylic acid **4** (101 mg, 72% yield calculated from **16**) as a white solid; $R_f = 0.26$ (EtOAc); mp: 103–105 °C; $[\alpha]_D^{23} = +17.1$ ($c = 0.9$ in $CHCl_3$); ^{1}H NMR (250.12 MHz, CD_3CN): $\delta = 7.93–7.90$ (m, 3H; 2Ar-*H*, O*H*), 7.81–7.77 (m, 2H; 2Ar-*H*), 7.73–7.60 (m, 2H; 2Ar-*H*), 7.58–7.18 (m, 3H; 3Ar-*H*, N*H*CS), 6.893–6.78 (m, 2H; N*H*Ac, N*H*CS), 5.104 (dd, $J_{2,3} = 10.6$ Hz, $J_{3,4} = 9.4$ Hz, 1H; H-3), 4.95 (dd, $J_{4,5} = 9.8$ Hz, 1H; H-4), 4.56 (d, $J_{1,2} = 8.6$ Hz, 1H; H-1), 4.22 (dd, $J_{5,6b} = 5.0$ Hz, $J_{6a,6b} = 12.3$ Hz, 1H; H-6b), 4.03 (dd, $J_{5,6a} = 2.5$ Hz, 1H; H-6a), 4.01 (d, $J_{vic} = 10.42$ Hz, 1H; *C*HNSO$_2$), 3.97–3.43 [m, 10H; H-2, H-5, 2*C*H$_2$NH, *C*H$_2$O, *C*H$_2$NSO$_2$), 2.18 (m, 1H; Me$_2$*C*H), 1.74 (m, 2H; *C*H$_2$), 1.99, 1.96, 1.94 (3s, each 3H; 3*Me*COO), 1.88 (s, 3H; *Me*CON), 0.95 (d, $J_{vic} = 6.6$ Hz, 3H; *Me*$_2$CH), 0.92 ppm (d, $J_{vic} = 6.5$ Hz, 3H; *Me*$_2$CH); ^{13}C NMR (62.9 MHz, CD_3CN): $\delta = 182.4$ (C=S), 172.8, 171.3, 171.2, 171.1, 170.5 (5C=O), 146.1 (Ar-*C*-SO$_2$), 139.9, 139.7, (2Ar-*C*), 130.0–128.2 (Ar-*C*H), 101.7 (C-1), 73.1 (C-3), 72.3 (C-5), 69.7 (C-4), 69.5 (*C*H$_2$O), 66.4 (*C*HNSO$_2$), 62.8 (C-6), 54.8 (C-2), 44.9, 44.8, 44.7 (2*C*H$_2$N, *C*H$_2$NSO$_2$), 29.4 (*C*H$_2$), 29.2 (*C*HMe$_2$), 23.2 (*Me*CON), 20.9 (3*Me*COO), 20.4, 19.7 ppm (*Me*$_2$CH); elemental analysis calcd (%) for $C_{37}H_{50}N_4O_{13}S_2$ (822.94): C 54.00, H 6.12, N 6.81; found: C 54.02, H 6.10, N 6.79.

Carboxylic acid 10: Isothiocyanate **19** (49.8 mg, 0.133 mmol) was solubilized in dry CH_2Cl_2/DMF (4:1, 13.5 mL), and a solution of amine **27** (89.4 mg, 0.133 mmol) in dry CH_2Cl_2/DMF (4:1, 13.5 mL) containing Et$_3$N (37 μL, 2 equiv) was added dropwise. The mixture was stirred at 60 °C for 20 h, until TLC analysis (EtOAc) indicated the formation of a major product ($R_f = 0.40$). The mixture was concentrated under reduced pressure, and the crude residue was taken up in Et$_2$O (10 mL) and washed with H$_2$O (10 mL). The aqueous layer was extracted with Et$_2$O (2×10 mL). The collected organic extracts were combined and dried (MgSO$_4$·H$_2$O), filtered, and concentrated under reduced pressure. The crude product (114 mg) was purified by flash chromatography (silica gel, *n*-hexane/EtOAc 2:8) to afford a white solid (80 mg) constituted (NMR) by ester **30** impure of isothiocyanates **19** (5%). Data for **30**: ^{1}H NMR (250.12 MHz, CD_3CN): $\delta = 7.88–7.84$ (m, 2H, Ar-H), 7.77–7.64 (m, 2H, Ar H), 7.61–7.57 (m, 2H, Ar H), 7.45–7.37 (m, 4H, Ar-H), 7.29 (brs, 1H, NHCS), 7.18 (brt, 1H, $J = 5.3$ Hz, NHCS),7.10–7.04 (m, 2H, Ar-H), 6.67 (d, 1H, $J_{2,NH} = 8.8$ Hz, NHAc), 5.24 (brs, 1H, H-1), 5.19 (dd, 1H, $J_{2,3} = 10.4$ Hz, $J_{3,4} = 9.3$ Hz, H-3), 5.12 (s, 2H, Ar*C*H$_2$O), 4.98

(dd, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 4.21 (dd, 1H, $J_{5,6b}$ = 4.7 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6b), 4.10–4.05 (m, 2H, H-2, H-6a), 3.84 (d, 1H, J_{vic} = 10.5 Hz, *CH*NSO_2), 3.98–3.71 [m, 4H, H-5, CH_2NH (1H), CH_2NSO_2], 3.35 m, 1H, CH_2NH (1H)], 2.22 (m, 1H, Me_2CH), 1.99, 1.97, 1.96 (3s, each 3H, 3*Me*COO), 1.86 (s, 3H, *Me*CON), 1.18 (s, 9H, CMe_3), 1.00 (d, 3H, J_{vic} = 6.4 Hz, Me_2CH), 0.91 ppm (d, 3H, J_{vic} = 6.6 Hz, Me_2CH); ^{13}C NMR (62.9 MHz, CD_3CN): δ = 184.3 (C=S), 172.8, 171.3, 171.2, 170.5, 170.4 (5C=O), 160.0 (Ar-*C*-O), 145.8 (Ar-*C*-SO_2), 138.2, 137.0, 134.1, 132.5 (4Ar-*C*), 130.2–128.0 (Ar-*C*H), 116.3 (2Ar-*C*H), 83.9 (C-1), 82.8 (Me_3CO), 73.4 (C-5), 73.2 (C-3), 69.5 (C-4), 69.8 (ArCH_2O), 67.6 (*CH*NSO_2), 62.8 (C-6), 55.6 (C-2), 45.9 *C*H_2NSO_2), 44.2 (*C*H_2NH), 29.8 (*CH*Me_2), 27.9 (Me_3C), 23.0 (*Me*CON), 20.9 (3*Me*COO), 20.5, 19.5 ppm (Me_2CH). Crude ester **30** (80 mg) was dissolved in CH_2Cl_2 (1.4 mL) and treated with CF_3COOH (0.54 mL), and the mixture was stirred at 0 °C for 1 h and then at RT. After 24 h, TLC analysis (EtOAc) revealed the complete disappearance of the starting material and the formation of a more retained product (R_f = 0.33). The solution was co-evaporated with toluene (4×10 mL) under reduced pressure, and trituration of the crude product with Et_2O afforded pure carboxylic acid **10** (52 mg, 43% yield calculated from **19**) as a white solid; R_f = 0.33 (EtOAc); mp: 119–120 °C; $[\alpha]_D^{23}$ = +12.6 (*c* = 0.9 in CH_3OH); ^{1}H NMR (250.12 MHz, CD_3CN): δ = 7.88–7.71 (m, 2H; 2Ar-*H*), 7.67–7.60 (m, 2H; 2Ar-*H*), 7.58–7.52 (m, 2H; 2Ar-*H*), 7.47–7.38 (m, 5H; 4Ar-*H*, OH), 7.34–7.17 (m, 2H; 2N*H*CS), 7.14–7.08 (m, 2H; Ar-*H*), 6.79 (d, $J_{2,NH}$ = 9.0 Hz, 1H; N*H*Ac), 5.41 (brs, 1H; H-1), 5.19 (dd, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 9.4 Hz, 1H; H-3), 5.11 (s, 2H; ArCH_2O), 4.97 (dd, $J_{4,5}$ = 10.0 Hz, 1H; H-4), 4.28 (dd, $J_{5,6b}$ = 4.4 Hz, $J_{6a,6b}$ = 12.4 Hz, 1H; H-6b), 3.98 (d, J_{vic} = 10.2 Hz, 1H; *CH*NSO_2), 4.10–3.60 (m, 5H; H-2, H-6a, H-5, *C*H_2NSO_2), 3.35 [m, 2H; *C*H_2NH), 2.17 (m, 1H; Me_2CH), 1.97, 1.96, 1.94 (3s, each 3H; 3*Me*COO), 1.84 (s, 3H; *Me*CON), 0.92 (d, J_{vic} = 6.6 Hz, 3H; Me_2CH), 0.89 ppm (d, J_{vic} = 6.7 Hz, 3H; Me_2CH); ^{13}C NMR (62.9 MHz, CD_3CN): δ = 182.9 (C=S), 173.7, 171.3, 171.2, 170.6, 170.5 (5C=O), 160.0 (Ar-*C*-O), 145.7 (Ar-*C*-SO_2), 137.9, 137.0, 134.1, 132.5 (4Ar-*C*), 130.2–127.8 (Ar-*C*H), 116.3 (2Ar-*C*H), 83.2 (C-1), 73.4 (C-5), 73.2 (C-3), 69.5 (C-4), 69.8 (ArCH_2O), 66.6 (*CH*NSO_2), 62.8 (C-6), 53.7 (C-2), 45.2 *C*H_2NSO_2), 44.4 (*C*H_2NH), 29.1 (*CH*Me_2), 23.0 (*Me*CON), 20.9 (3*Me*COO), 20.3, 19.6 ppm (Me_2CH); elemental analysis calcd (%) for $C_{41}H_{49}ClN_4O_{13}S_2$ (905.43): C 54.39, H 5.45, N 6.19; found: C 54.37, H 5.43, N 6.17.

General procedure for the synthesis of deprotected carboxylic acids 3, 5, 7, 9, and 11: A solution of protected carboxylic acid **2**, **4**, **6**, **8**, or **10** (0.1 mmol) in MeOH (1 mL) was treated with 7 N NH_3/MeOH (1 mL), and the solution was stirred at RT until the starting compound was completely reacted (TLC, $CHCl_3$/MeOH 8:2, 20–24 h). The solution was co-evaporated with toluene (4×10 mL) under reduced pressure, and crystallization (MeOH/EtOAc) or trituration of the crude product with Et_2O afforded the pure carboxylic acid.

Carboxylic acid 3: Trituration with Et_2O afforded pure **3** (94% yield) as a white solid; R_f = 0.25 ($CHCl_3$/MeOH 8:2); mp: 131–133 °C; $[\alpha]_D^{23}$ = +20.3 (*c* = 1.01 in MeOH); ^{1}H NMR (250.12 MHz, CD_3OD/D_2O): δ = 7.96–7.92 (m, 2H; 2Ar-*H*), 7.81–7.75 (m, 2H; 2Ar-*H*), 7.64 (m, 2H; 2Ar-*H*), 7.49–7.39 (m, 3H; 3Ar-*H*), 4.20–3.25 (m, 12H; H-1, H-2, H-3, H-4, H-5, H-6a, H-6b, *CH*NSO_2, *C*H_2NSO_2, *C*H_2NHCS), 2.19 (m, 1H; Me_2CH), 1.93 (s, 3H; *Me*CON), 0.96 ppm (m, 6H; Me_2CH); ^{13}C NMR (62.9 MHz, CD_3OD/D_2O): δ = 183.1 (C=S), 174.9,174.8 (2C=O), 146.7 (Ar-*C*-SO_2), 140.3, 139.0 (2Ar-*C*), 129.9–128.1 (Ar-*C*H), 84.3 (C-1), 79.4 (C-5), 75.7 (C-3), 71.7 (C-4), 66.8 (*C*HN), 62.5 (C-6), 55.9 (C-2), 46.1, 44.4 (*C*H_2NH, *C*H_2NSO_2), 29.5 (*CH*Me_2), 22.8 (*Me*CON), 20.6, 19.9 ppm (Me_2CH); elemental analysis calcd (%) for $C_{28}H_{38}N_4O_9S_2$ (638.75): C 52.65, H 6.00, N 8.77; found: C 52.63, H 6.01, N 8.76.

Carboxylic acid 5: Trituration with Et_2O afforded pure **5** (88% yield) as a white solid; R_f = 0.07 ($CHCl_3$/MeOH 8:2); mp: 114–116 °C; $[\alpha]_D^{23}$ = −4.0 (*c* = 1.08 in $CHCl_3$); ^{1}H NMR (250.12 MHz, CD_3OD/D_2O): δ = 7.99–7.94 (m, 2H; 2Ar-*H*), 7.78–7.74 (m, 2H; 2Ar-*H*), 7.68–7.65 (m, 2H; 2Ar-*H*), 7.49–7.38 (m, 3H; 3Ar-*H*), 4.39 (d, $J_{1,2}$ = 8.5 Hz, 1H; H-1), 4.08–3.78 [m, 5H; H-6b, *C*H_2NSO_2, *CH*NSO_2, *C*H_2O (1H)], 3.76–3.59 [m, 4H; H-2, H-6a, *C*H_2O (1H), *C*H_2NH (1H)], 3.58–3.30 [m, 6H; H-3, H-4, H-5, *C*H_2NH (1H), *C*H_2NH (2H)], 1.98 (s, 3H; *Me*COON), 1.75 (m, 2H; *C*H_2), 0.98 (brd, J_{vic} = 6.8 Hz, 3H; Me_2CH), 0.90 ppm (brs, 3H; Me_2CH); ^{13}C NMR (62.9 MHz, CD_3OD/D_2O): δ = 179.8 (C=S), 174.2 174.1 (2C=O), 146.4 (Ar-*C*-SO_2), 140.4, 140.2 (2Ar-*C*), 130.1–128.2 (Ar-*C*H), 102.5 (C-1), 77.5 (C-5), 75.8 (C-3), 71.9 (C-4), 70.7 (*CH*NSO_2), 67.9 (*C*H_2O), 62.5 (C-6), 57.2 (C-2), 43.5, 43.0, 42.8 (*C*H_2NSO_2, 2*C*H_2NH), 30.0 (Me_2CH), 29.8 (CH_2), 23.2 (*Me*CON), 20.4 ppm (2Me_2*CH*); elemental analysis calcd (%) for $C_{31}H_{44}N_4O_{10}S_2$ (696.83): C 53.43, H 6.36, N 8.04; found: C 53.41, H 6.33, N 8.01.

Carboxylic acid 7: Crystallization (MeOH/EtOAc) afforded pure **7** (67% yield) as a white solid; R_f = 0.13 ($CHCl_3$/MeOH 8:2); mp: 115–117 °C; $[\alpha]_D^{23}$ = −10.2 (*c* = 1.2 in MeOH); ^{1}H NMR (250.12 MHz, CD_3OD/D_2O): δ = 7.87–7.27 (m, 10H; 9Ar-*H*, *CH*-triazole), 5.87 (brs, 1H; H-1), 4.35–3.15 (m, 9H; H-2, H-3, H-4, H-5, H-6a, H-6b, *C*H_2N, *CH*N,), 2.08 (m, 1H; Me_2CH), 1.82 (s, 3H; *Me*COON), 0.90–0.78 ppm (m, 6H; Me_2CH); ^{13}C NMR (62.9 MHz, CD_3OD/D_2O): δ = 176.6,173.6 (2C=O), 146.4 (Ar-*C*-SO_2, *C*-triazole), 140.4, 139.7 (2Ar-*C*), 130.4–128.5 (Ar-*C*H), 121.8 (*C*H-triazole), 87.8 (C-1), 80.8 (C-5), 75.4 (C-3), 70.9 (C-4), 62.0 (*C*HN), 61.9 (C-6), 56.7 (C-2), 40.4 (*C*H_2NSO_2), 25.4 (*CH*Me_2), 23.1 (*Me*CON), 21.0, 20.0 ppm (Me_2CH); elemental analysis calcd (%) for $C_{28}H_{35}N_5O_9S$ (617.67): C 54.45, H 5.71, N 11.34; found: C 54.43, H 5.70, N 11.32.

Carboxylic acid 9: Trituration with Et_2O afforded pure **9** (70% yield) as a white solid; R_f = 0.17 ($CHCl_3$/MeOH 8:2); mp: 156–157 °C; $[\alpha]_D^{23}$ = +12.3 (*c* = 1.08 in MeOH); ^{1}H NMR (250.12 MHz, CD_3OD/D_2O): δ = 7.87–7.27 (m, 10H; 9Ar-*H*, *CH*-triazole), 4.32 (d, $J_{1,2}$ = 8.3 Hz, 1H; H-1), 4.25–3.10 (m, 13H; H-2, H-3, H-4, H-5, H-6a, H-6b, *C*H_2NSO_2, *CH*N, *C*H_2N, *C*H_2O), 2.18–203. (m, 3H; *C*H_2, Me_2CH), 1.89 (s, 3H; *Me*COON), 1.02–0.81 ppm (m, 6H; Me_2CH);^{13}C NMR (62.9 MHz, CD_3OD/D_2O): δ = 175.3 170.5 (2C=O), 146.3 (Ar-*C*-SO_2, *C*-triazole), 140.4 (2Ar-*C*), 130.3–129.1 (Ar-*C*H), 128.3 (*C*H-triazole), 102.7 (C-1), 77.9 (C-5), 75.9 (C-3), 72.0 (C-4), 66.9 (*C*H_2O), 66.7 (*C*HN), 62.7 (C-6), 57.3 (C-2), 47.9 (*C*H_2N), 41.8 (*C*H_2NSO_2), 30.7 (CH_2), 30.6 (*CH*Me_2), 23.4–22.0 ppm (*Me*CON, Me_2CH); elemental analysis calcd (%) for $C_{31}H_{41}N_5O_{10}S$ (675.75): C 55.10, H 6.12, N 10.36; found: C 55.08, H 6.10, N 10.34.

Carboxylic acid 11: Trituration with Et_2O afforded pure **11** (79% yield) as a white solid; R_f = 0.10 ($CHCl_3$/MeOH 8:2); mp: 134–136 °C; $[\alpha]_D^{23}$ = +27.7 (*c* = 0.85 in $CHCl_3$); ^{1}H NMR (250.12 MHz, CD_3OD/D_2O): δ = 7.98–7.82 (m, 2H; 2Ar-*H*), 7.77–7.73 (m, 2H; 2Ar-*H*), 7.66–7.62 (m, 2H; 2Ar-*H*), 7.45–7.38 (m, 4H; 4Ar-*H*), 7.12–7.08 (m, 2H; 2Ar-*H*), 5.12 (s, 2H; ArCH_2O), 4.05–3.31 (m, 12H; H-1, H-2, H-3, H-4, H-5, H-6a, H-6b, *CH*NSO_2, *C*H_2NSO_2, *C*H_2NHCS), 2.18 (m, 1H, Me_2CH), 1.97 (s, 3H; *Me*CON), 1.03–0.97 ppm (m, 6H; Me_2CH); ^{13}C NMR (62.9 MHz, CD_3OD/D_2O): δ = 181.7 (C=S), 174.8, 173.0 (2C=O), 160.4 (Ar-*C*-O), 146.2 (Ar-*C*-SO_2), 138.4, 137.3, 134.5, 133.1 (4Ar-*C*), 129.6–127.8 (Ar-*C*H), 116.5 (2Ar-*C*H), 84.5 (C-1), 78.9 (C-5), 75.8 (C-3), 71.8, (C-4), 70.2 (*CH*NSO_2), 68.7 (ArCH_2O), 62.6 (C-6), 56.1 (C-2), 45.9, 44.8 (*C*H_2NH, *C*H_2NSO_2), 29.6 (Me_2CH), 22.8 (*Me*CON), 20.7, 19.9 ppm (Me_2CH); elemental analysis calcd (%) for $C_{35}H_{43}ClN_4O_{10}S_2$ (779.32): C 53.94, H 5.56, N 7.19; found: C 53.91, H 5.53, N 7.17.

Biological methods

MMP inhibition assays: Recombinant human MMP-14 catalytic domain was a kind gift from Prof. Gillian Murphy (Department of Oncology, University of Cambridge, UK). Pro-MMP-1, pro-MMP-2, and pro-MMP-9 were purchased from Calbiochem (Merck-Millipore). Pro-MMP-12 was purchased from R&D Systems. Proenzymes were activated immediately prior to use with *p*-aminophenylmercuric acetate (2 mM APMA for 1 h at 37 °C for MMP-2, 2 mM APMA for 2 h at 37 °C for MMP-1, and 1 mM APMA for 1 h at 37 °C for MMP-9). Pro-MMP-12 was autoactivated by incubating in fluorimetric 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, the inhibitor stock solutions (DMSO, 10 mM) were further diluted in FAB. Activated enzyme (final concentrations of 0.56 nM for MMP-2, 1.3 nM for MMP-9, 1.0 nM for MMP-14 cd, 2.0 nM for MMP-1, 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 duplicate in a total volume of 200 μL per well in 96-well microtiter plates (Corning black, NBS). Control wells lacked 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} values were determined by using Equation (1):

$$\frac{v_i}{v_0} = \frac{1}{(1 + [I]/IC_{50})} \quad (1)$$

in which v_i is the initial velocity of substrate cleavage in the presence of the inhibitor at concentration [I] and v_0 is the initial velocity in the absence of the inhibitor. Results were analyzed by using SoftMax Pro software and Prism Software version 5.0 (GraphPad Software, Inc., La Jolla, CA).

Protein preparation: The expression and purification for the catalytic domains used in the crystallographic studies, MMP-9 (MMP9wt) and MMP12 (hMMP12wt) and the active-site glutamate to glutamine mutant hMMP12 (E219Q), were described elsewhere.[28] In brief, the synthetic gene used for the catalytic domain of human MMP-9 comprised residues Gly106–Gly215 and Gln391–Gly444, without the fibronectin domains, those for MMP-12. Plasmids were propagated in the *Escherichia coli* strain XL1-Blue, and the recombinant catalytic domains expressed in *E. coli* cells BL21 (DE3 star). After induction, the cells were harvested by centrifugation, the pellets were resuspended, and the cells were suspended, disrupted, centrifuged, and redissolved in 8 M urea. The protein was then refolded, purified and dialyzed as previously described.[28] Acetohydroxamic acid (AHA) in the range 10–120 mM was added to prevent self-degradation during concentration.

Crystallization: The crystallization trials were performed by sitting vapor diffusion with 1 μL equivolumetric drops of protein–substrate and reservoir solution using CrysChem plates stored in a cooled incubator at 20 °C. Initial screening for the MMP-12 glycoconjugate complexes was performed with five precipitant solutions: 27% polyethylene glycol 10000 (PEG-10 K), 0.2 M imidazole malate, pH 8.5; 45% (PEG-4 K), 0.2 M imidazole piperidine, pH 8.5; 17% PEG-20 K, 0.2 M imidazole malate, pH 8.5, 250 mM NaCl; 27% PEG-10 K, 150 mM imidazole piperidine, pH 8.5 and 17% PEG-20 K, 250 mM NaCl, 100 mM Tris-HCl, pH 10.0 used in the crystallization of other MMP-12 ligand complexes but with insufficient precipitating power for the MMP-12 glycoconjugate complexes. These precipitants were supplemented with 5–20% dioxane (Table S1, see the Supporting Information). Crystals remained small and crystal growth was so slow that to avoid excessive protein degradation the E219Q MMP-12 mutant was used instead of the more active wild-type enzyme for several MMP-12 complexes. Crystals for the trigonal polymorph of MMP-9 in complex with compound **2** were obtained from 40% monomethyl PEG 5000 (MPEG-5 K), 100 mM bicine, pH 7.25 (Table S1), conditions similar to those used for co-crystals with the hydroxamate-based inhibitor ARP101.[29]

Cryoprotection and data collection: The crystals used for data collection were picked up with a litho-loop from the crystallization tray and were transferred to cryoprotectant solutions, which in the case of MMP-9 also contained 1 mM compound **2** (Table S1) to prevent the loss of the ligand during the cryosoak phase of the experiment. The cryoprotectant solutions were prepared with mixed components either from 40% *v/v* CryoSol or CryoProtX (Molecular Dimensions, UK) solutions, 50% *v/v* precipitant, and 10% *v/v* buffer as previously described.[30] Crystals were soaked in these solutions (Table S1) for a few seconds, then picked up into a loop, and finally flash-cooled in liquid nitrogen by using magnetic SPINE compatible cryovials for data collection at the ESRF synchrotron facility or transferred to Unipucks (MiTeGen, USA) for robotic mounting. Data were collected either on beamlines Proxima 1 or Proxima 2A[31] at the Soleil synchrotron Facility (St. Aubin, France) or at The ESRF synchrotron facility (Grenoble, France) on the user-operated beamline ID23–2 or dispatched to the fully automatic ID30A beamline. In all cases, data were collected from a single crystal at 100 K with MxCube[32] and were reduced by using XDS[33] and XSCALE by using the script "xdsme" (https://github.com/legrandp/xdsme).

Crystallographic structure determination: The structures were solved by molecular replacement by using phaser[34] or MOLREP[35] and were refined by using REFMAC5[36] and Phenix.[37] The MMP-9 crystal belongs to the space group $P3_221$ diffracting to 1.59 Å resolution.

Model building and electron density interpretation

The ligands were built with the CCP4 ligand sketcher[38] and fitted into the difference electron density (weighted F_0-F_c) calculated and displayed by using COOT.[39] The electronic density for the inhibitor portion of the compounds was of excellent quality, whereas the sugar moiety was poorly defined for most of the copies of the ligand in the asymmetric unit. The presence of the sugar moiety on the inhibitor shifted the preference for the $P2_12_12$ space group with a single molecule in the asymmetric unit to a monoclinic $P2_1$ cell with two molecules in the asymmetric unit, a = 39.1 Å b = 62.9 Å c = 63.7 Å, β = 102.5° for the MMP-12·**2** complex or four molecules in a larger cell a = 63.7 Å b = 63.1 Å c = 78.9 Å, β = 103.1° for the MMP-12·**3** complex and with similar cell parameters for the other complexes. The packing of the MMP-12 catalytic domain in the larger cell was related to that in the smaller one. A strong pseudo-translational noncrystallographic (NCS) operation related the two cells with a Patterson off-origin peak that was 38.6% of the origin peak. The sugar moiety of the inhibitor was positioned at a crystal contact and was the likely cause of the larger cell.

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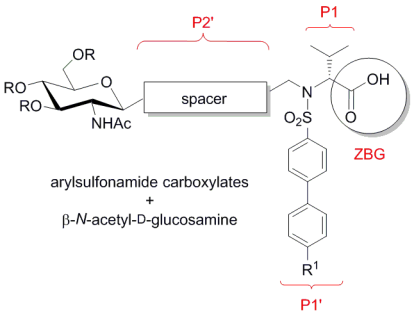
Sugar power: Ten glycoconjugate arylsulfonamide carboxylates were designed and synthesized as matrix metalloproteinase-12 (MMP-12)-selective inhibitors with improved hydrophilicity. They were tested on recombinant MMPs by fluorimetric assays, and X-ray crystallographic studies helped rationalize the results. Introduction of β-*N*-acetyl-D-glucosamine at the P2′ position was found to maintain nanomolar activity against MMP-12 and to boost bioavailability.



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Sugar-Based Arylsulfonamide Carboxylates as Selective and Water-Soluble Matrix Metalloproteinase-12 Inhibitors