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Ferrier sulfamidoglycosylation of glycals catalyzed by nitrosonium tetrafluoroborate: Towards new carbonic anhydrase glycoinhibitors

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ABSTRACT

Ferrier sulfamidoglycosylation of glycals catalyzed by nitrosonium tetrafluoroborate allowed the preparation of hydroxysulfamide glycosides in good yields with a good α stereoselectivity. A variety of monosaccharide derivatives was synthesized using this new methodology leading to selective and powerful glycoinhibitors of the tumor associated carbonic anhydrases (CA, EC 4.2.1.1) isoforms CA IX and CA XII.

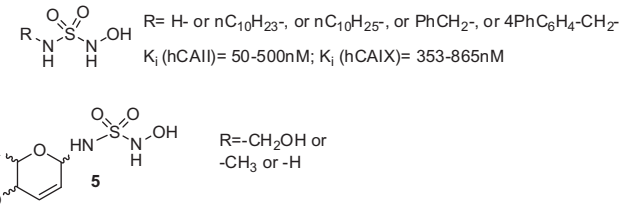
1. Introduction

The development of carbonic anhydrases (CA, EC 4.2.1.1) glycoinhibitors is currently a dynamic field which constitutes one of the most successful approaches to find new active and selective inhibitors with potent biomedical applications.[1] Carbohydrate based CA inhibitors were already found to be effective as antiglaucoma or anti-epileptic agents.[1] Some recent work from our group also demonstrated the validity of this approach in the field of cancer as we were able to demonstrate that carbonic anhydrase inhibitors belonging to a glycosylcoumarin series were strong, selective CA IX inhibitors and were able to reduce the growth of primary tumors and metastases in a human and mouse model of orthotopic, CA IX-positive breast cancer[2] and also to deplete cancer stem cells within these tumors.[3] In our ongoing researches in the development of new and original glycoinhibitors incorporating the hydroxysulfamide scaffold as zinc binding function,[4] we were interested to find a synthetic methodology allowing the access to 2,3-unsaturated glycosides **5** starting from a peracetylated glycals platform (Scheme 1).

Based on previous works reported by Colinas and Bravo[5] describing the Ferrier sulfonamidoglycosylation of glycals, we sought to extend this methodology to sulfamides using the non-metallic catalyst nitrosyl tetrafluoroborate. Indeed, $NOBF_4$ was recently demonstrated by Misra and Coll.[6] to be efficient for the stereoselective glycosylation reaction as well as for the preparation of 2,3-unsaturated glycosides and 2-deoxyglycosides. In this paper, we report our findings on this reaction and also the inhibitory activity of the obtained *N*-glycosyl-*N*-hydroxysulfamides against four relevant CA isoforms.

2. Results and discussion

We first focused our attention to the conditions for the Ferrier sulfamidoglycosylation of the tri-*O*-acetyl-D-glucal with *N*-(*O*-*tert*-butyldiphenylsilyl)hydroxysulfamide **1**, prepared initially from the commercial hydroxylamine hydrochloride, following a



Scheme 1. Structure of hydroxysulfamide CA inhibitors previously described[4] and general structure of the targeted compound **5**.

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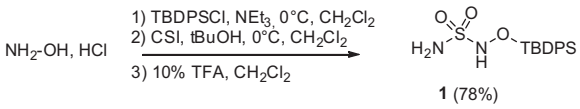


Scheme 2. Synthesis of *N*-(*O*-*tert*-butyldiphenylsilyl)hydroxysulfamide **1**.

three steps synthesis as depicted in Scheme 2. The hydroxylamine was first O-protected with the *tert*-butyldiphenylsilyl protecting group, and then sulfamoylated with *tert*-butoxysulfamoyl chloride, prepared ab initio by reacting chlorosulfonyl isocyanate with *tert*-butanol. Final Boc deprotection with 10% TFA solution in methylene chloride allowed formation of compound **1** in an overall yield of 78%.[7]

First attempts of sulfamidoglycosylation of glycal **2** was realized using the classical Lewis acid $BF_3.Et_2O$, widely used as catalyst in Ferrier rearrangement of glycals and described in 2007 by Colinas research group as a good catalyst of the Ferrier sulfonamidoglycosylation of D-glycals.[5] Unfortunately, low reaction yields (around 20%), obtained with this catalyst, led us to use $NOBF_4$, recently reported as mild, efficient and inexpensive catalyst for the Ferrier rearrangement reaction.[6]

In order to improve in term of efficiency, selectivity, time and yield of reaction we investigated the reaction in the presence of a variable amount of $NOBF_4$, at different temperatures. We found that the reaction proceeded effectively at 50 °C using 1.1 equiv of **1** and 0.05 equiv of $NOBF_4$ in methylene chloride. Other organic solvents were tested (e.g., acetonitrile, chloroform), but no improvements in terms of yields and reaction time were observed.

The reaction was also subjected to microwave irradiation instead of thermal activation. In this case, an overall 50% reduction of the reaction time was possible.

These optimized conditions were applied on a variety of glycals such as D-galactal, D-arabinal, L-arabinal and L-rhamnal. (Scheme 3) and the corresponding Ferrier sulfamidoglycosylation products **3** were obtained in moderate to fairly good yields. In all cases, a mixture of non-separable α- and β-anomers was obtained, with the α-anomer being predominant in all cases (Table 1), as it was observed also in the case of sulfonamidoglycosylation reactions reported by Colinas and Bravo.[5]

Removal of the silyl protecting groups from compound **3** was initially performed by standard methods using either tetra-*n*-butyl ammonium fluoride (TBAF)[8], or Selectfluor™[9] and led to low yields (~20%) of the corresponding compound **4**. Ultimately deprotection of the silyl ether was carried out using HF–pyridine complex and

Table 1
Optimized results for the Ferrier sulfamidoglycosylation and deprotection steps

Glycals **1**	Step 1[a] Yield %/α/β ratio/reaction time	Steps 2+3[b] Yield %/α/β ratio
2a	Δ: 68/60:40/45 min MW: 70/60:40/20 min	85/77:23
2b	Δ: 70/75:25/45 min MW: 70/75:25/20 min	85/77:23
2c	Δ: 88/60:40/45 min MW: 90/60:40/20 min	85/77:23
2d	Δ: 86/60:40/45 min MW: 90/60:40/20 min	85/77:23
2e	Δ: 88/84:16/45 min MW: 90/84:16/20 min	85/77:23

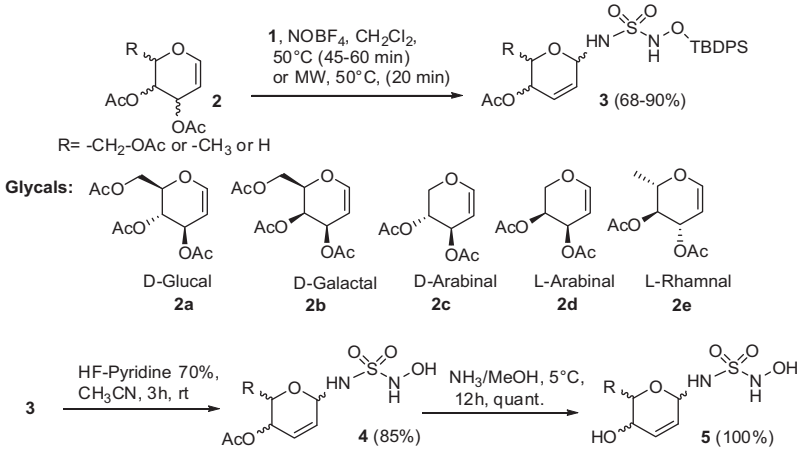
[a] Reaction conditions: glycal (**1**) 1 equiv *N*-(*O*-*tert*-butyldiphenylsilyl)hydroxysulfamide 1.1 equiv, $NOBF_4$ 0.05 equiv, CH_2Cl_2.
[b] HF-pyridine 70%, CH_3CN, RT, then NH_3/MeOH.

compound **4** were obtained in 85% yields.[10] Final cleavage of the acetyl group was done by using a methanolic solution of ammonia, to afford quantitatively compound **5**.

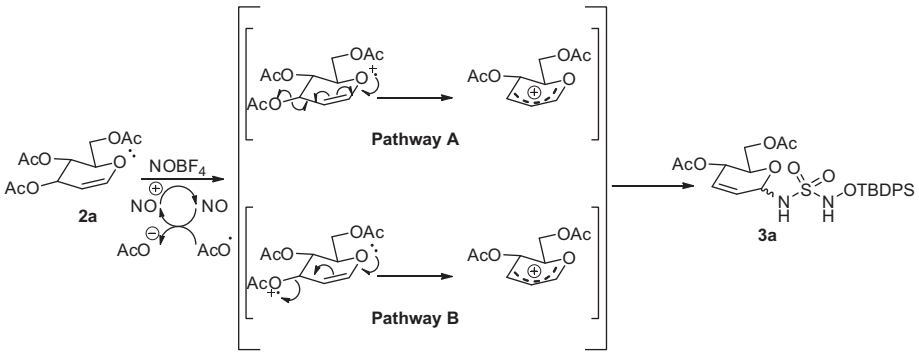
The structure of compounds **1**, **3**, **4** and **5**, as well as the ratio of α/β anomers were unambiguously confirmed using ^1H, ^{13}C, 2D COSY pulseprog COSYGPQF (with gradient quadrature mode; time domain size, TD = 2 k; relaxation delay, d1 = 1.5 s and scan number = 1), HMQC experiments.

Stereochemical assignment of the major diastereoisomer was verified by NOESY pulseprog NOESYGPPHPP experiments (time domain size, TD = 2 k; the mixing time d8 = 0.7 s; the relaxation delay d1 = 1.5 s and scans number = 16), observing a NOE interaction between H_1 and H_5 for the β-anomers (absent for the α anomers).

At this point, we can provide two plausible mechanisms for the sulfamidoglycosylation reaction of glycals, inspired by previous work reported by Ansari et al.[11] and Toshima et al.[12] These mechanisms are depicted in Scheme 4 and illustrated in the case of the peracetylated glucal **2a**. In the first step of these mechanism one electron coming or from the ring oxygen (pathway A) or from the oxygen of the acetate group located on position 3 (pathway B) could be accepted by the nitrosonium cation to give an acetate radical (which in turn could accept an electron from NO to give the acetate anion). Then the delocalized carbocation could undergone nucleophilic displacement reaction by the *N*-(*O*-*tert*-butyldiphenylsilyl)hydroxysulfamide **1** with allylic rearrangement leading to the 2,3-unsaturated glycoside **3a** as a mixture of α and β anomers, α anomer being predominant.



Scheme 3. Synthesis of compound **5** via the Ferrier sulfamidoglycosylation.



Scheme 4. Proposed mechanisms for the sulfamidoglycosylation reaction/example of Glucal.

Table 2
Inhibitory activity of compounds **4a**–**4e** and **5a**–**5e** against the four CA isoforms: hCA I, II, IX and XII determined by a Stopped-Flow, CO_2 Hydration Assay Method.[13] Selectivity ratios for the inhibition of the tumor-associated (CA IX and XII) over the cytosolic (CA II) isozyme are also reported

	K_I[a] (nM)				Selectivity ratio	
	hCA I[b]	hCA II[b]	hCA IX[c]	hCA XII[c]	K_I hCA II/K_I hCA IX	K_I hCA II/K_I hCA XII
AAZ	250	12	25	5.6	0.48	2.14
4a	813	94	90	76	1.04	1.23
4b	752	86	79	207	1.08	0.41
4c	>10,000	>10,000	54	86	>185	>116
4d	>10,000	>10,000	44	81	>227	>123
4e	>10,000	94	47	93	2	1.01
5a	>10,000	91	9	93	10.11	0.97
5b	917	>10,000	44	68	>227	>147
5c	927	>10,000	8	88	>1250	>113
5d	>10,000	85	9	415	9.44	0.20
5e	>10,000	>10,000	8	87	>1250	>114

[a] Errors in the range of ±5–10% of the reported value from three different determinations.
[b] Full length, cytosolic isoform.
[c] Catalytic domain, recombinant enzyme.

3. Carbonic anhydrase inhibition assays

The compounds reported here were investigated for the inhibition of four human (h) CA isoforms, involved in crucial physiologic processes in mammals, the cytosolic, widespread hCA I and II, as well as the tumor associated, transmembrane isoforms hCA IX and XII. Inhibition data with compounds **4** and **5**, as well as the sulfonamide in clinical use acetazolamide (as standard compound) are reported in Table 2.

It may be observed that the slow cytosolic isoforms (hCA I) was poorly or not at all inhibited by the compounds reported here. Just **4a**, **4b**, and **5b**, **5c** showed inhibition constants around 1000 nM, whereas all other derivatives were not inhibitory against hCA I up to 10 μM. hCA II showed a very unusual inhibition profile with the new derivatives. In fact two peracetylated compounds (**4c** and **4d**) and three deacetylated ones (**5b**, **5c** and **5e**) were not inhibitory up to concentrations of 10 μM, whereas the remaining ones showed medium potency inhibitory action, with K_Is in the range of 85–94 nM. Thus, in this case the nature of the glycal strongly influences activity but in manner difficult to rationalize considering both the acetylated as well as the deacetylated series.

The tumor associated hCA IX on the other hand was effectively inhibited by all the new compounds, with the acetylated derivatives **4** being slightly less effective (K_Is in the range of 44–90 nM) than the deacetylated ones, **5**, which had K_Is in the range of 8–44 nM. Generally the nature of the glycal was not very important for the inhibitory power except galactal which led to a derivative around one order of magnitude less potent compared to the other sugar derivatives investigated here. hCA XII was moderately inhibited by both compounds **4** and **5** reported here, with K_Is in the range of 68–415 nM, with two compounds in each series (**4b** and **5d**, respectively) showing the weakest inhibition (K_Is of 207 and 415 nM, respectively) whereas the remaining ones showed a quite compact behavior of medium potency inhibitor (K_Is in the range of 68–93 nM).

Some of the new compounds reported here, such as **4c**, **4d**, **5b**, **5c** and **5e** showed profiles of significant tumor-associated CAs selective inhibitors, with selectivity ratios of >100 for inhibiting hCA IX/XII over hCA I/II.

4. Conclusion

In conclusion, our methodology allowed the preparation of hydroxysulfamide glycosides from glycals in good yields and with a good alpha stereoselectivity. We have used an effective sulfamidoglycosylation method using $NOBF_4$ as a catalyst in dichloromethane. Studies on the inhibitory activity against the two cytosolic CA isoforms: hCA I, II, and the two tumor associated, membrane isoforms hCA IX and XII demonstrated the importance of the sugar scaffold in the design of powerful and selective hCA IX/hCA XII inhibitors with potential in antitumor therapy, although the structure–activity relationship in this small series of inhibitors is not at all straightforward.

5. Experimental section

5.1. General methods

Microwaves synthesis was carried out on CEM Discover Microwaves of CEM Corporation. The thin layer chromatographies (TLC)

were performed on silica plates Merck 60 F354 aluminum. Reactions were monitored by TLC by using alumina plates coated with silica gel and visualized either by using UV light or by charring with of 10% sulfuric acid in ethanol solution. Column chromatography was performed on silica gel 60 Å, particle size: 35–70 mesh. ^{1}H and ^{13}C NMR spectra were recorded on Brucker-400 instrument using the residual solvent signals as an internal reference. High-resolution mass spectra (HRMS) were obtained from an ESI-TOF-MS spectrometer (SYNAPT G2-S of Waters).

5.2. Microwave irradiation experiments

All microwave experiments were performed with CEM Discover Synthesizer possessing (Sp). On this device, the temperature is controlled via an optical fiber directly inside the reactor, providing greater accuracy in the measurements. Experiments were carried out in standard microwaves process vials 10 ml capacity (filled with 7 ml max). The specifications used are pressure: 17 bars; power: 150–200 W; temperature: 50 °C and power max: off.

5.3. Synthesis

5.3.1. *N*-(*O*-*tert*-Butyldiphenylsilyl)hydroxysulfamide (1)

Triethylamine (5 mL, 37 mmol) was added dropwise to a suspension of hydroxylamine hydrochloride (1 g, 14.4 mmol) in anhydrous CH_2Cl_2 (20 mL) under N_2 at 0 °C. The mixture was stirred at room temperature for 2 h. then cooled to 0 °C. A solution of *tert*-butyldiphenylsilyl chloride TBDPSCl (1.80 g, 12 mmol) in anhydrous CH_2Cl_2 (10 mL) was then slowly added at 0 °C. The reaction mixture was stirred at room temperature for 20 h. In a separate flask, *tert*-butanol (1.14 mL, 12 mmol) was added dropwise to a solution of chlorosulfonylisocyanate (1.02 mL, 12 mmol) in anhydrous CH_2Cl_2 (30 mL) under N_2 at 0 °C and stirred at 0 °C for 40 min to give the *tert*-butoxycarbamoylsulfamoyl chloride. Triethylamine (5 mL, 37 mmol) was added to the solution of protected hydroxylamine at 0 °C and the solution containing the *tert*-butoxycarbamoylsulfamoyl chloride was added dropwise via a syringe. The reaction mixture was warmed to rt and stirred for 18 h. The solvent was removed under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed with water (10 mL), 0.1 M HCl (3 × 10 mL), satd $NaHCO_3$ (3 × 10 mL), brine (15 mL). The organic layer was finally dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue obtained was then dissolved and stirred at 0 °C in a solution of 10% TFA–CH_2Cl_2. The reaction was monitored by TLC until the complete disappearance of starting material. The solvent was removed under reduced pressure to give the expected compound **1** in 78% yield.

Mp = 110 °C; R_f = 0.30 (100% CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (s, 1H, NH), 7.70–7.79 (m, 4H, 4× CH_{ortho}), 7.43–7.48 (m, 2H, 2× CH_{para}), 7.36–7.43 (m, 4H, 4× CH_{meta}), 6.95 (s, 2H, NH_2), 1.07 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (101 MHz, DMSO-d_6) δ 135.5 (CH_{ortho}), 132.4 (CSi), 129.7 (CH_{para}), 127.4 (CH_{meta}), 26.8 ($C(CH_3)_3$), 19.1 ($C(CH_3)_3$). MS ESI *m*/*z* 351.15 $[M+H]^+$.

5.3.2. General procedure for the synthesis of compound 3

To a solution of peracetylated glycal (1 mmol) and *N*-(*O*-*tert*-butyldiphenylsilyl)hydroxysulfamide (1.1 mmol, 1.1 equiv) in 5 ml of dry CH_2Cl_2 was added $NOBF_4$ (0.05 mmol, 0.05 equiv). The mixture was then, or stirred under reflux (50 °C) or stirred under microwaves irradiations until completion of the reaction. The reaction mixture was concentrated under reduced pressure and the crude product was purified on silica gel using diethyl ether and pentane as eluent (**3a**, **3b** and **3e**: Et_2O/pentane 5:5; **3c** and **3d** Et_2O/pentane 6:4). Compound **3** are obtained as a mixture of α/β isomers.

5.3.2.1. Compound 3a. We introduced 0.272 g of **2a** and we obtained 0.383 g (yield Δ: 68%) and 0.394 g (MW: 70%). HRMS calcd for $C_{26}H_{35}N_2O_8SSi$ $[M+H]^+$: 563.1883, found 563.1880 (−0.3 mDa).

*5.3.2.1.1. N-[1-(4,6-Di-O-acetyl-2,3-dideoxy-α-D-erythro-hex-2-eno-pyranosyl)]-N-(O-tert-butyldiphenylsilyl)sulfamide (**3a**, anomer α, 60%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H, NHOSi), 9.01 (d, 1H, *J* = 8.6 Hz, NHCH), 7.74–7.66 (m, 4H, 4× CH_{ortho}), 7.50–7.44 (m, 2H, 2× CH_{para}), 7.43–7.38 (m, 4H, 4× CH_{meta}), 5.90 (td, *J* = 10.2, 1.6 Hz, 1H, H-3), 5.82 (ddd, *J* = 10.2, 2.9, 2.1 Hz, 1H, H-2), 5.37 (ddd, *J* = 8.6, 2.9, 1.6 Hz 1H, H-1), 5.20–5.16 (m, 1H, H-4), 4.15 (dd, *J* = 11.6, 2.5 Hz, 1H, H-6a), 4.11–4.09 (m, 1H, H-5), 4.03 (dd, *J* = 11.6, 4.3 Hz, 1H, H-6b), 2.06 (s, 3H, CH_3COO), 1.98 (s, 3H, CH_3COO), 1.06 (s, 9H, 3× CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.9 (C═O), 169.6 (C═O), 135.5 (CH_{ortho}), 131.9 (CSi), 130.2 (CH_{para}), 128.4 (C-3), 127.6 (C-2), 127.3 (CH_{meta}), 76.2 (C-1), 66.8 (C-5), 64.1 (C-4), 62.3 (C-6), 26.61 ($C(CH_3)_3$), 20.59 (CH_3COO), 20.29 (CH_3COO), 18.93 ($C(CH_3)_3$).

*5.3.2.1.2. N-[1-(4,6-Di-O-acetyl-2,3-dideoxy-β-D-threo-hex-2-eno-pyranosyl)]-N-(O-tert-butyldiphenylsilyl)sulfamide (**3a**, anomer β, 40%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H, NHOSi), 8.88 (d, *J* = 8.6 Hz, 1H, NHCH), 7.74–7.66 (m, 4H, 4× CH_{ortho}), 7.50–7.44 (m, 2H, 2× CH_{para}), 7.43–7.38 (m, 4H, 4× CH_{meta}), 5.87 (td, *J* = 10.2, 1.6 Hz, 1H, H-3), 5.86 (td, *J* = 10.2, 1.3 Hz, 1H, H-2), 5.44–5.39 (m, 1H, H-1), 5.24–5.20 (m, 1H, H-4), 4.15 (dd, *J* = 11.6, 2.5 Hz, 1H, H-6a), 4.05 (dd, *J* = 11.6, 4.3 Hz, 1H, H-6b), 3.86 (ddd, *J* = 8.25, 4.74, 3.24 Hz, 1H, H-5), 2.04 (s, 3H, CH_3COO), 1.97 (s, 3H, CH_3COO), 1.07 (s, 9H, 3× CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.8 (C═O), 169.6 (C═O), 135.5 (CH_{ortho}), 132.0 (CSi), 130.2 (CH_{para}), 129.8 (C-3), 127.8 (C-2), 127.3 (CH_{meta}), 78.8 (C-1), 73.2 (C-5), 63.9 (C-4), 62.6 (C-6), 26.61 ($C(CH_3)_3$), 20.59 (CH_3COO), 20.31 (CH_3COO), 18.87 ($C(CH_3)_3$).

5.3.2.2. Compound 3b. We introduced 0.272 g of **2b** and we obtained 0.394 g (yield Δ: 70%) and 0.394 g (MW: 70%). HRMS calcd for $C_{26}H_{35}N_2O_8SSi$ $[M+H]^+$: 563.1883, found 563.1880 (−0.3 mDa).

*5.3.2.2.1. N-[1-(4,6-Di-O-acetyl-2,3-dideoxy-α-D-threo-hex-2-eno-pyranosyl)]-N-(O-tert-butyldiphenylsilyl)sulfamide (**3b**, anomer α, 75%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (s, 1H, NHOSi), 8.90 (d, *J* = 9.0 Hz, 1H, N*H*CH), 7.75–7.65 (m, 4H, 4× CH_{ortho}), 7.50–7.44 (m, 2H, 2× CH_{para}), 7.44–7.37 (m, 4H, 4× $CH_{méta}$), 6.06 (ddd, *J* = 9.9, 5.4, 1.4 Hz, 1H, H-3), 6.02 (dd, *J* = 9.9, 3.2 Hz, 1H, H-2), 5.39 (ddd, *J* = 9.0, 3.0, 1.3 Hz, 1H, H-1), 4.96 (dd, 1H, H-4), 4.40 (dd, *J* = 6.5, 2.5 Hz, 1H, H-5), 4.14 (dd, *J* = 11.2, 6.5 Hz, 1H, H-6a), 4.00 (dd, *J* = 11.2, 6.5 Hz, 1H, H-6b), 2.01 (s, 3H, CH_3COO), 1.93 (s, 3H, CH_3COO), 1.07 (s, 9H, 3× CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.2 (C═O), 170.1 (C═O), 135.9 (*C*H$_{ortho}$), 132.3 (CSi), 130.8 (C-2), 130.2 (*C*H$_{para}$), 127.8 (*C*H$_{meta}$), 124.9 (C-3), 76.3 (C-1), 67.1 (C-5), 62.2 (C-4), 62.0 (C-6), 27.03 (C(*C*H$_3$)$_3$), 20.7 (2× *C*H$_3$COO), 19.4 (*C*(CH$_3$)$_3$).

*5.3.2.2.2. N-[1-(4,6-Di-O-acetyl-2,3-dideoxy-β-D-erythro-hex-2-eno-pyranosyl)]-N-(O-tert-butyldiphenylsilyl)sulfamide (**3b**, anomer β, 25%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H, NHOSi), 9.00 (d, *J* = 9.4 Hz, 1H, N*H*CH), 7.75–7.65 (m, 4H, 4× CH_{ortho}), 7.50–7.44 (m, 2H, 4× CH_{para}), 7.44–7.37 (m, 4H, 4× CH_{meta}), 6.04 (ddd, *J* = 10.0, 5.0, 1.8, 1H, H-3), 5.99 (br d, *J* = 10.0 Hz, 1H, H-2), 5.31 (d, *J* = 8.2 Hz, 1H, H-1), 5.05 (td, *J* = 5.0, 1.8 Hz, 1H, H-4), 4.09–4.11 (m, 2H, H-6a, H-6b), 4.03 (dt, *J* = 6.2, 2.4 Hz, 1H, H-5), 2.02 (s, 3H, CH_3COO), 1.97 (s, 3H, CH_3COO), 1.07 (s, 9H, 3× CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.4 (C═O), 170.3 (C═O), 135.9 (*C*H$_{ortho}$), 132.3 (CSi), 130.8 (C-2), 130.3 (*C*H$_{para}$), 127.8 (*C*H$_{meta}$), 124.9 (C-3), 79.4 (C-1), 72.3 (C-5), 63.0 (C-4), 62.5 (C-6), 26.99 (C(*C*H$_3$)$_3$), 20.81 (*C*H$_3$COO), 20.76 (*C*H$_3$COO), 19.34 (*C*(CH$_3$)$_3$).

5.3.2.3. Compound 3c. We introduced 0.200 g of **2c** and we obtained 0.431 g (yield Δ: 88%) and 0.441 g (MW: 90%). HRMS calcd for $C_{23}H_{31}N_2O_6SSi$ $[M+H]^+$: 491.1672, found 491.1675 (0.3 mDa).

*5.3.2.3.1. N-[1-(4-O-Acetyl-5-anhydro-2,3-dideoxy-α-D-erythro-pent-2-enopyranosyl)]-N-(O-tert-butyldiphenylsilyl)sulfamide (**3c**, anomer α, 60%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H, NHOSi), 8.86 (d, *J* = 9.0 Hz, 1H, N*H*CH), 7.74–7.67 (m, 4H, 4× CH_{ortho}), 7.50–7.44 (m, 2H, 2× CH_{para}), 7.44–7.37 (m, 4H, 4× CH_{meta}), 6.04 (tdd, *J* = 9.9, 4.9, 1.3 Hz, 1H, H-3), 5.99–5.96 (m, 1H, H-2), 5.35–5.31 (m, 1H, H-1), 4.90–4.84 (m, 1H, H-4), 4.18 (dd, *J* = 13.1, 1.3 Hz, 1H, H-5a), 3.74 (dd, *J* = 13.1, 1.3 Hz, 1H, H-5b), 2.03 (s, 3H, CH_3COO), 1.07 (s, 9H, 3× CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.2 (C=O), 135.9 (*C*H$_{ortho}$), 132.3 (CSi), 130.9 (C-2), 130.2 (*C*H$_{para}$), 127.79 (*C*H$_{meta}$), 125.1 (C-3), 75.5 (C-1), 63.2 (C-4), 61.8 (C-5), 26.95 (C(*C*H$_3$)$_3$), 21.04 (*C*H$_3$COO), 19.3 (*C*(CH$_3$)$_3$).

*5.3.2.3.2. N-[1-(4-O-Acetyl-5-anhydro-2,3-dideoxy-β-D-threo-pent-2-enopyranosyl)]-N-(O-tert-butyldiphenylsilyl)sulfamide (**3c**, anomer β, 40%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.15 (s, 1H, NHOSi), 8.96 (d, *J* = 9.0 Hz, 1H, N*H*CH), 7.74–7.67 (m, 4H, 4× CH_{ortho}), 7.50–7.44 (m, 2H, 2× CH_{para}), 7.44–7.37 (m, 4H, 4× CH_{meta}), 6.01–5.99 (m, 1H, H-3), 5.88 (ddd, *J* = 10.1, 2.2, 1.3 Hz, 1H, H-2), 5.26 (dd, *J* = 9.0, 2.0 Hz, 1H, H-1), 5.10–5.04 (m, 1H, H-4), 3.81 (dd, *J* = 11.8, 4.9 Hz, 1H, H-5a), 3.80 (dd, *J* = 11.8, 5.8 Hz, 1H, H-5b), 2.03 (s, 3H, CH_3COO), 1.07 (s, 9H, 3× CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.3 (C=O), 135.9 (*C*H$_{ortho}$) 132.3 (CSi), 130.6 (C-2), 130.2 (*C*H$_{para}$), 127.8 (C-3), 127.8 (*C*H$_{meta}$), 77.2 (C-1), 64.1 (C-4), 62.2 (C-5), 26.98 (C(*C*H$_3$)$_3$), 21.0 (CH_3COO), 19.3 (*C*(CH$_3$)$_3$).

5.3.2.4. Compound 3d. We introduced 0.200 g of **2d** and we obtained 0.421 g (yield Δ: 86%) and 0.441 g (MW: 90%). HRMS calcd for $C_{23}H_{31}N_2O_6SSi$ $[M+H]^+$: 491.1672, found 491.1672 (0.0 mDa).

*5.3.2.4.1. N-[1-(4-O-Acetyl-5-anhydro-2,3-dideoxy-α-L-erythro-pent-2-enopyranosyl)]-N-(O-tert-butyldiphenylsilyl)sulfamide (**3d**, anomer α, 60%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H, NHOSi), 8.86 (d, *J* = 9.0 Hz, 1H, N*H*CH), 7.75–7.67 (m, 4H, 4× CH_{ortho}), 7.50–7.44 (m, 2H, 2× CH_{para}), 7.44–7.38 (m, 4H, 4× CH_{meta}), 6.04 (tdd, *J* = 10.1, 4.9, 1.4 Hz, 1H, H-3), 6.02–5.99 (m, 1H, H-2), 5.33 (dd, *J* = 9.0, 1.8 Hz, 1H, H-1), 4.89–4.84 (m, 1H, H-4), 4.18 (dd, *J* = 13.2, 2.9 Hz, 1H, H-5a), 3.74 (dd, *J* = 13.2, 1.4 Hz, 1H, H-5b), 2.03 (s, 3H, CH_3COO), 1.07 (s, 9H, 3× CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.3 (C=O), 135.87 (*C*H$_{ortho}$), 132.26 (CSi), 130.9 (C-2), 130.2 (*C*H$_{para}$), 127.80 (*C*H$_{meta}$), 125.1 (C-3), 75.5 (C-1), 63.2 (C-4), 61.8 (C-5), 26.96 (C(*C*H$_3$)$_3$), 21.05 (*C*H$_3$COO), 19.30 (*C*(CH$_3$)$_3$).

*5.3.2.4.2. N-[1-(4-O-Acetyl-5-anhydro-2,3-dideoxy-β-L-thréo-pent-2-enopyranosyl)]-N-(O-tert-butyldiphenylsilyl)sulfamide (**3d**, anomer β, 40%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.15 (s, 1H, NHOSi), 8.95 (d, *J* = 9.0 Hz, 1H, N*H*CH), 7.75–7.67 ((m, 4H, 4× CH_{ortho}), 7.50–7.44 (m, 2H, 2× CH_{para}), 7.44–7.38 (m, 4H, 4× CH_{meta}), 5.99–5.97 (m, 1H, H-3), 5.90 (ddd, *J* = 10.1, 2.1, 1.3 Hz, 1H, H-2), 5.26 (dd, *J* = 9.0, 1.9 Hz, 1H, H-1), 5.12–5.03 (m, 1H, H-4), 3.82 (dd, *J* = 11.7, 6.0 Hz, 1H, H-5a), 3.79 (dd, *J* = 11.7, 4.7 Hz, 1H, H-5b), 2.03 (s, 2H), 2.03 (s, 3H, CH_3COO), 1.08 (s, 9H, 3× CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.2 (C=O), 135.85 (*C*H$_{ortho}$), 132.3 (CSi), 130.6 (C-2), 130.1 (*C*H$_{para}$), 127.82 (C-3), 127.80 (*C*H$_{meta}$), 77.2 (C-1), 64.1 (C-4), 62.2 (C-5), 26.98 (C(*C*H$_3$)$_3$), 21.00 (CH_3COO), 19.30 (C(*C*H$_3$)$_3$).

5.3.2.5. Compound 3e. We introduced 0.214 g of **2e** and we obtained 0.444 g (yield Δ: 88%) and 0.454 g (MW: 90%). HRMS calcd for $C_{24}H_{33}N_2O_6SSi$ $[M+H]^+$: 505.1829 found 505.1826 (−0.3 mDa).

*5.3.2.5.1. N-[1-(4-O-Acetyl-6-anhydro-2,3-dideoxy-α-L-threo-hex-2-enopyranosyl)]-N-(O-tert-butyldiphenylsilyl)sulfamide (**3e**, anomer α, 84%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (s, 1H, NHOSi), 8.78 (d, *J* = 9.2 Hz, 1H, NHCH), 7.74–7.68 (m, 4H, 4× CH_{ortho}), 7.50–7.44 (m, 2H, 2× CH_{para}), 7.44–7.37 (m, 4H, 4× CH_{meta}), 5.84–5.80 (m, 2H, H-2, H-3), 5.34 (br s, 1H, H-1), 4.90–4.88 (m, 1H, H-4), 3.92 (d, *J* = 6.2 Hz, 1H, H-5), 2.07 (s, 3H, CH_3), 1.08 (s, 9H, 3× CH_3), 1.07 (s, 3H, CH_3COO). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.0 (C=O), 135.6 (CH_{ortho}), 132.1 (CSi), 130.3 (C-2), 129.9 (CH_{para}), 128.9 (C-3), 127.5 (CH_{meta}), 78.8 (C-1), 71.7 (C-5), 69.4 (C-4), 26.8 ($C(CH_3)_3$), 20.8 (CH_3COO), 19.1 ($C(CH_3)_3$), 18.3 (CH_3).

*5.3.2.5.2. N-[1-(4-O-Acetyl-6-anhydro-2,3-dideoxy-β-L-erythro-hex-2-enopyranosyl)]-N-(O-tert-butyldiphenylsilyl)sulfamide (**3e**, anomer β, 16%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H, NHOSi), 8.97 (d, *J* = 8.9 Hz, 1H, NHCH), 7.74–7.68 (m, 4H, 4× CH_{ortho}), 7.50–7.44 (m, 2H, 2× CH_{para}), 7.44–7.37 (m, 4H, 4× CH_{meta}), 5.90–5.85 (m, 2H, H-2, H-3), 5.32 (br s, 1H, H-1), 4.88–4.85 (m, 1H, H-4), 3.94 (d, *J* = 6.2 Hz, 1H, H-5), 2.07 (s, 3H, CH_3), 1.08 (s, 9H, 3× CH_3), 1.07 (s, 3H, CH_3COO). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.0 (C=O), 135.6 (CH_{ortho}), 132.1 (CSi), 130.3 (C-2), 129.9 (CH_{para}), 128.9 (C-3), 127.5 (CH_{meta}), 76.2 (C-1), 69.6 (C-4), 65.0 (C-5), 26.8 ($C(CH_3)_3$), 20.8 (CH_3COO), 19.1 ($C(CH_3)_3$), 18.3 (CH_3).

5.3.3. General procedure for the synthesis of compound 4

To a solution of compound **3** (0.63 mmol) in 7 ml of CH_3CN was added 1.7 equiv of HF·pyr complex (70%) at room temperature for 3 h. The reaction was then quenched by addition of Et_3N (5 ml) and the mixture was concentrated under vacuum. The crude product was then purified by silica gel column chromatography using diethyl ether and pentane as eluent (**4a**, **4b** and **4e**: Et_2O/pentane 7:3; **4c** and **4d** Et_2O/pentane 8:2). The expected compound **4** (mixture α/β) are obtained in 85% yield.

5.3.3.1. Compound 4a. We introduced 0.353 g of **3a** and we obtained 0.173 g (yield: 85%). HRMS calcd for $C_{10}H_{17}N_2O_8S$ $[M+H]^+$: 325.0706, found 325.0703 (−0.3 mDa).

*5.3.3.1.1. N-[1-(4,6-Di-O-acetyl-2,3-dideoxy-α-D-erythro-hex-2-eno-pyranosyl)]-N-hydroxysulfamide (**4a**, anomer α, 77%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.92 (s, 1H, NHOH), 6.69 (s, 1H, NHCH), 5.87 (td, *J* = 10.5, 1.8 Hz, 1H, H-3), 5.79 (ddd, *J* = 10.5, 3.2, 2.5 Hz, 1H, H-2), 5.24 (ddd, *J* = 9.2, 2.9, 1.8 Hz, 1H, H-1), 5.12–5.10 (m, 1H, H-4), 4.12 (dd, *J* = 12.0, 4.1 Hz, 1H, H-6a), 4.12–4.08 (m, 1H, NHOH), 4.08–4.05 (m, 1H, H-5), 4.03 (dd, *J* = 11.6, 4.3 Hz, 1H, H-6b), 2.05 (s, 3H, CH_3COO), 2.02 (s, 3H, CH_3COO). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.0 (C=O), 169.8 (C=O), 130.4 (s, 1H, C-3), 127.0 (C-2), 87.5 (C-1), 65.8 (C-5), 64.8 (C-4), 62.8 (C-6), 20.4 (CH_3COO).

*5.3.3.1.2. N-[1-(4,6-Di-O-acetyl-2,3-dideoxy-β-D-threo-hex-2-eno-pyranosyl)]-N-hydroxysulfamide (**4a**, anomer β, 23%).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.90 (s, 1H, NHOH), 6.68 (s, 1H, NHCH), 5.84 (td, *J* = 10.5, 1.6 Hz, 1H, H-3), 5.79 (td, *J* = 10.5, 1.3 Hz, 1H, H-2), 5.33–5.28 (m, 1H, H-1), 5.14–5.12 (m, 1H, H-4), 4.12–4.08 (m, 3H, NHOH, H-6a, H-6b), 3.89 (ddd, *J* = 8.2, 4.7, 3.2 Hz, 1H, H-5), 2.04 (s, 3H, CH_3COO), 2.03 (s, 3H, CH_3COO). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.0 (C=O), 169.8 (C=O), 132.9 (C-3), 126.0 (C-2), 90.3 (C-1), 72.1 (C-5), 64.3 (C-4), 62.9 (C-6), 20.6 (CH_3COO).

5.3.3.2. Compound 4b. We introduced 0.357 g of **3b** and we obtained 0.175 g (yield: 85%). HRMS calcd for $C_{10}H_{17}N_2O_8S$ $[M+H]^+$: 325.0706, found 325.0703 (−0.3 mDa).

*5.3.3.2.1. N-[1-(4,6-Di-O-acetyl-2,3-dideoxy-α-D-threo-hex-2-eno-pyranosyl)]-N-hydroxysulfamide (**4b**, anomer α, 77%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.92 (s, 1H, NHOH), 6.69 (s, 1H, NHCH), 6.07 (ddd, *J* = 9.9, 5.3, 1.4 Hz, 1H, H-3), 6.02 (dd, *J* = 9.9, 3.3 Hz, 1H, H-2), 5.42–5.38 (m, 1H, H-1), 4.96 (dd, *J* = 5.2, 2.5 Hz, 1H, H-4), 4.40 (dd, *J* = 6.5, 2.5 Hz, 1H, H-5), 4.13–4.10 (m, 1H, NHOH), 4.09 (dd, *J* = 12.0, 4.1 Hz, 1H, H-6a), 4.06 (dd, *J* = 11.2, 6.5 Hz, 1H, H-6b), 1.95 (s, 6H, 2× CH_3COO). ^{13}C NMR (101 MHz, DMSO-d_6) δ

169.8 (C=O), 130.6 (C-2), 127.3 (C-3), 87.5 (C-1), 65.9 (C-5), 63.0 (C-4), 62.7 (C-6), 20.4 (2× CH_3COO).

*5.3.3.2.2. N-[1-(4,6-Di-O-acetyl-2,3-dideoxy-β-D-erythro-hex-2-eno-pyranosyl)]-N-hydroxysulfamide (**4b**, anomer β, 23%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.90 (s, 1H, NHOH), 6.68 (s, 1H, NHCH), 6.05 (ddd, J = 10.1, 5.1, 1.6 Hz, 1H, H-3), 6.00 (br d, J = 10.5 Hz, 1H, H-2), 5.34 (dd, J = 9.0, 4.7 Hz, 1H, H-1), 4.96 (dd, J = 5.0, 1.8 Hz, 1H, H-4), 4.13–4.10 (m, 3H, NHOH, H-6a, H-6b), 4.08 (dt, J = 6.2, 2.4 Hz, 1H, H-5), 2.00 (s, 6H, 2× CH_3COO). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.0 (C=O), 133.1 (C-2), 126.1 (C-3), 90.3 (C-1), 72.27 (C-5), 64.9 (C-4), 64.4 (C-6), 20.6 (2× CH_3COO).

5.3.3.3. Compound 4c. We introduced 0.309 g of **3c** and we obtained 0.135 g (yield: 85%). HRMS calcd for $C_7H_{13}N_2O_6S$ $[M+H]^+$: 253.0494, found 253.0492 (−0.2 mDa).

*5.3.3.3.1. N-[1-(4-O-Acetyl-5-anhydro-2,3-dideoxy-α-D-erythro-pent-2-enopyranosyl)]-N-hydroxysulfamide (**4c**, anomer α, 77%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.92 (s, 1H, NHOH), 6.69 (s, 1H, NHCH), 6.07–6.02 (m, 1H, H-3), 5.99–5.96 (m, 1H, H-2), 5.36–5.33 (m, 1H, H-1), 4.90–4.84 (m, 1H, H-4), 4.22–4.13 (m, 2H, H-5a, H-5b), 3.85–3.69 (m, 1H, NHOH), 2.03 (s, 3H, CH_3COO). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.2 (C=O), 130.9 (C-2), 125.1 (C-3), 87.6 (C-1), 63.2 (C-4), 61.8 (C-5), 21.04 (CH_3COO).

*5.3.3.3.2. N-[1-(4-O-Acetyl-5-anhydro-2,3-dideoxy-β-D-threo-pent-2-enopyranosyl)]-N-hydroxysulfamide (**4c**, anomer β, 23%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.90 (s, 1H, NHOH), 6.68 (s, 1H, NHCH), 6.07–6.02 (m, 1H, H-3), 5.88 (ddd, J = 10.1, 2.2, 1.3 Hz, 1H, H-2), 5.26 (dd, J = 9.0, 2.0 Hz, 1H, H-1), 4.90–4.84 (m, 1H, H-4), 3.85–3.69 (m, 3H, H-5a, H-5b, NHOH), 2.03 (s, 3H, CH_3COO). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.3 (C=O), 130.6 (C-2), 127.8 (C-3), 90.5 (C-1), 64.1 (C-4), 62.2 (C-5), 21.0 (CH_3COO).

5.3.3.4. Compound 4d. We introduced 0.311 g of **3d** and we obtained 0.136 g (yield: 85%). HRMS calcd for $C_7H_{13}N_2O_6S$ $[M+H]^+$: 253.0494, found 253.0494 (0.0 mDa).

*5.3.3.4.1. N-[1-(4-O-Acetyl-5-anhydro-2,3-dideoxy-α-L-threo-pent-2-enopyranosyl)]-N-hydroxysulfamide (**4d**, anomer α, 77%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.92 (s, 1H, NHOH), 6.69 (s, 1H, NHCH), 6.08–6.01 (m, 1H, H-3), 6.00–5.97 (m, 1H, H-2), 5.35 (dd, J = 9.0, 1.8 Hz, 1H, H-1), 4.89–4.84 (m, 1H, H-4), 4.22–4.13 (m, 2H, H-5a, H-5b), 3.84–3.70 (m, 1H, NHOH), 2.04 (s, 3H, CH_3COO). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.2 (C=O), 131.0 (C-2), 125.2 (C-3), 87.4 (C-1), 63.3 (C-4), 61.9 (C-5), 21.05 (CH_3COO).

*5.3.3.4.2. N-[1-(4-O-Acetyl-5-anhydro-2,3-dideoxy-β-L-erythro-pent-2-enopyranosyl)]-N-hydroxysulfamide (**4d**, anomer β, 23%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.90 (s, 1H, NHOH), 6.68 (s, 1H, NHCH), 6.08–6.01 (m, 1H, H-3), 6.00–5.97 (m, 1H, H-2), 5.27 (dd, J = 9.0, 2.0 Hz, 1H, H-1), 4.88–4.85 (m, 1H, H-4), 3.84–3.70 (m, 2H, H-5a, H-5b, NHOH), 2.03 (s, 3H, CH_3COO). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.3 (C=O), 130.3 (C-2), 127.8 (C-3), 90.3 (C-1), 64.2 (C-4), 62.2 (C-5), 21.00 (CH_3COO).

5.3.3.5. Compound 4e. We introduced 0.318 g of **3e** and we obtained 0.143 g (yield: 85%). HRMS calcd for $C_8H_{15}N_2O_6S$ $[M+H]^+$: 267.0651, found 267.0654 (0.3 mDa).

5.3.3.5.1. N-[1-(4-O-Acetyl-6-anhydro-2,3-dideoxy-α-L-erythro-hex-2-enopyranosyl)]-N-hydroxysulfamide (4e, anomer α, 77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.92 (s, 1H, NHOH), 6.69 (s, 1H, NHCH), 5.90–5.85 (m, 2H, H-2, H-3), 5.32 (br s, 1H, H-1), 4.88–4.85 (m, 1H, H-4), 3.98–3.89 (m, 2H, H-5, NHOH), 2.07 (s, 3H, CH_3), 1.07 (s, 3H, CH_3COO). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.0 (C=O), 130.2 (C-2), 127.5 (C-3), 87.4 (C-1), 69.6 (C-4), 65.1 (C-5), 20.8 (CH_3COO), 19.1 (CH_3).

*5.3.3.5.2. N-[1-(4-O-Acetyl-6-anhydro-2,3-dideoxy-β-L-threo-hex-2-enopyranosyl)]-N-hydroxysulfamide (**4e**, anomer β, 23%).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.90 (s, 1H, NHOH), 6.68 (s, 1H, NHCH), 5.84–5.80 (m, 2H, H-2, H-3), 5.34 (br s, 1H, H-1), 4.90–4.88 (m, 1H, H-4), 3.98–3.89 (m, 2H, H-5, NHOH), 2.07 (s, 3H, CH_3), 1.07 (s, 3H, CH_3COO). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.0 (C=O), 130.2 (C-2), 127.5 (C-3), 90.3 (C-1), 69.6 (C-4), 65.1 (C-5), 20.8 (CH_3COO), 19.1 (CH_3).

5.3.4. General procedure for the synthesis of compound 5

Compound **4** (0.318 mmol) were dissolved in a 2.0 M NH_3 methanolic solution (8 ml) at 0–5 °C. The reaction was stirred at 4 °C overnight, then concentrated under vacuum to give compound **5** (α and β anomers mixture) in quantitative yield.

5.3.4.1. Compound 5a. We introduced 0.103 g of **4a** and we obtained 0.076 g (yield: 100%). HRMS calcd for $C_6H_{13}N_2O_6S$ $[M+H]^+$: 241.0494, found 241.0496 (0.2 mDa).

*5.3.4.1.1. N-[1-(2,3-Dideoxy-α-D-erythro-hex-2-enopyranosyl)]-N-hydroxysulfamide (**5a**, anomer α, 77%).* ^{1}H NMR (400 MHz, D_2O) δ 6.92 (s, 1H, NHOH), 6.69 (s, 1H, NHCH), 5.90–5.88 (m, 1H, H-3), 5.85–5.83 (m, 1H, H-2), 5.29 (ddd, J = 9.4, 3.5, 1.7 Hz, 1H, H-1), 4.22 (dd, J = 8.8, 2.2 Hz, 1H, H-4), 3.89–3.87 (m, 4H, H-5, H-6a, H-6b, NHOH), 3.70–3.65 (m, 2H, OH). ^{13}C NMR (101 MHz, D_2O) δ 130.4 (C-3), 127.0 (C-2), 87.5 (C-1), 78.1 (C-5), 68.3 (C-4), 60.0 (C-6).

*5.3.4.1.2. N-[1-(2,3-Dideoxy-β-D-threo-hex-2-eno-pyranosyl)]-N-hydroxysulfamide (**5a**, anomer β, 23%).* ^{1}H NMR (400 MHz, D_2O) δ 6.90 (s, 1H, NHOH), 6.68 (s, 1H, NHCH), 5.90–5.88 (m, 1H, H-3), 5.85–5.83 (m, 1H, H-2), 5.32–5.26 (m, 1H, H-1), 4.24 (dd, J = 7.7, 1.5 Hz, 1H, H-4), 3.89–3.87 (m, 4H, H-5, H-6a, H-6b, NHOH), 3.70–3.65 (m, 2H, OH). ^{13}C NMR (101 MHz, D_2O) δ 130.35 (C-3), 127.02 (C-2), 90.34 (C-1), 78.11 (C-5), 68.78 (C-4), 60.03 (C-6).

5.3.4.2. Compound 5b. We introduced 0.103 g of **4b** and we obtained 0.077 g (yield: 100%). HRMS calcd for $C_6H_{13}N_2O_6S$ $[M+H]^+$: 241.0494, found 241.0496 (0.2 mDa).

*5.3.4.2.1. N-[1-(2,3-Dideoxy-α-D-threo-hex-2-eno-pyranosyl)]-N-hydroxysulfamide (**5b**, anomer α, 77%).* ^{1}H NMR (400 MHz, D_2O) δ 6.92 (s, 1H, NHOH), 6.69 (s, 1H, NHCH), 5.90–5.88 (m, 1H, H-3), 5.85–5.83 (m, 1H, H-2), 5.26–5.22 (m, 1H, H-1), 4.22 (dd, J = 6.0, 2.1 Hz, 1H, H-4) 3.93–3.88 (m, 4H, H-5, H-6a, H-6b, NHOH), 3.70–3.65 (m, 2H, OH). ^{13}C NMR (101 MHz, D_2O) δ 130.2 (C-3), 127.8 (C-2), 88.3 (C-1), 78.0 (C-5), 68.2 (C-4), 60.1 (C-6).

*5.3.4.2.2. N-[1-(2,3-Dideoxy-β-D-erythro-hex-2-eno-pyranosyl)]-N-hydroxysulfamide (**5b**, anomer β, 23%).* ^{1}H NMR (400 MHz, D_2O) δ 6.90 (s, 1H, NHOH), 6.68 (s, 1H, NHCH), 5.90–5.88 (m, 1H, H-3), 5.85–5.83 (m, 1H, H-2), 5.33–5.28 (m, 1H, H-1), 4.24 (dd, J = 6.2, 1.9 Hz, 1H, H-4), 3.93–3.88 (m, 4H, H-5, H-6a, H-6b, NHOH), 3.70–3.65 (m, 2H, OH). ^{13}C NMR (101 MHz, D_2O) δ 130.2 (C-3), 127.8 (C-2), 91.0 (C-1), 78.0 (C-5), 68.8 (C-4), 60.1 (C-6).

5.3.4.3. Compound 5c. We introduced 0.080 g of **4c** and we obtained 0.067 g (yield: 100%). HRMS calcd for $C_5H_{11}N_2O_5S$ $[M+H]^+$: 211.0389, found 211.0386 (0.3 mDa).

*5.3.4.3.1. N-[1-(5-Anhydro-2,3-dideoxy-α-D-erythro-pent-2-eno-pyranosyl)]-N-hydroxysulfamide (**5c**, anomer α, 77%).* ^{1}H NMR (400 MHz, D_2O) δ 7.32 (s, 1H, NHOH), 7.09 (s, 1H, NHCH), 5.98–5.96 (m, 1H, H-3), 5.96–5.94 (m, 1H, H-2), 5.47–5.45 (m, 1H, H-1), 4.61–4.59 (m, 1H, H-4), 4.18–3.99 (m, 3H, H-5a, H-5b, NHOH), 3.55 (br s, 1H, NHOH). ^{13}C NMR (101 MHz, D_2O) δ 130.8 (C-2), 127.6 (C-3), 88.0 (C-1), 64.0 (C-4), 63.3 (C-5).

*5.3.4.3.2. N-[1-(5-Anhydro-2,3-dideoxy-β-D-threo-pent-2-enopyranosyl)]-N-hydroxysulfamide (**5c**, anomer β, 23%).* ^{1}H NMR (400 MHz, D_2O) δ 7.30 (s, 1H, NHOH), 7.08 (s, 1H, NHCH), 5.98–5.96 (m, 1H, H-3), 5.96–5.94 (m, 1H, H-2), 5.44–5.43 (m, 1H, H-1), 4.65–4.64 (m, 1H, H-4), 4.18–3.99 (m, 3H, H-5a, H-5b, NHOH), 3.55 (br s, 1H, NHOH). ^{13}C NMR (101 MHz, D_2O) δ 130.8 (C-2), 127.6 (C-3), 92.0 (C-1), 64.0 (C-4), 63.3 (C-5).

5.3.4.4. Compound 5d. We introduced 0.080 g of **4d** and we obtained 0.067 g (yield: 100%). HRMS calcd for $C_5H_{12}N_2O_5S$ $[M+H]^+$: 211.0389, found 211.0388 (−0.1 mDa).

*5.3.4.4.1. N-[1-(5-Anhydro-2,3-dideoxy-α-L-threo-pent-2-eno-pyranosyl)]-N-hydroxysulfamide (**5d**, anomer α, 77%).* ^{1}H NMR (400 MHz, D_2O) δ 7.29 (s, 1H, NHOH), 6.99 (s, 1H, NHCH), 5.95–5.90 (m, 1H, H-3), 5.89–5.85 (m, 1H, H-2), 5.38–5.35 (m, 1H, H-1), 4.69–4.63 (m, 1H, H-4), 4.21–4.13 (m, 3H, H-5a, H-5b, NHOH), 3.86 (br s, 1H, NHOH). ^{13}C NMR (101 MHz, D_2O) δ 130.52 (C-2), 127.64 (C-3), 87.16 (C-1), 66.09 (C-4), 63.42 (C-5).

*5.3.4.4.2. N-[1-(5-Anhydro-2,3-dideoxy-β-L-erythro-pent-2-eno-pyranosyl)]-N-hydroxysulfamide (**5d**, anomer β, 23%).* ^{1}H NMR (400 MHz, D_2O) δ 7.26 (s, 1H, NHOH), 6.96 (s, 1H, NHCH), 5.95–5.90 (m, 1H, H-3), 5.89–5.85 (m, 1H, H-2), 5.38–5.35 (m, 1H, H-1), 4.63–451 (m, 1H, H-4), 4.21–4.13 (m, 3H, H-5a, H-5b, NHOH), 3.86 (br s, 1H, NHOH). ^{13}C NMR (101 MHz, D_2O) δ 130.52 (C-2), 127.76 (C-3), 91.54 (C-1), 66.09 (C-4), 63.42 (C-5).

5.3.4.5. Compound 5e. We introduced 0.085 g of **4e** and we obtained 0.072 g (yield: 100%). HRMS calcd for $C_6H_{13}N_2O_5S$ $[M+H]^+$: 225.0545, found 225.0543 (−0.2 mDa).

*5.3.4.5.1. N-[1-(6-Anhydro-2,3-dideoxy-α-L-threo-hex-2-eno-pyranosyl)]-N-hydroxysulfamide (**5e**, anomer α, 77%).* ^{1}H NMR (400 MHz, D_2O) δ 6.92 (s, 1H, NHOH), 6.69 (s, 1H, NHCH), 6.07 (m, 1H, H-3), 6.02–5.98 (m, 1H, H-2), 5.41–5.39 (m, 1H, H-1), 4.45–4.40 (m, 1H, H-4), 3.98–3.89 (m, 2H, H-5, NHOH), 2.00 (br s, 3H, CH_3). ^{13}C NMR (101 MHz, D_2O) δ 130.1 (C-2), 126.9 (C-3), 87.4 (C-1), 70.5 (C-4), 66.0 (C-5), 19.6 (CH_3).

*5.3.4.5.2. N-[1-(6-Anhydro-2,3-dideoxy-β-L-erythro-hex-2-eno-pyranosyl)]-N-hydroxysulfamide (**5e**, anomer β, 23%).* We introduced 0.085 g of **4e** and we obtained 0.072 g (yield: 100%). ^{1}H NMR (400 MHz, D_2O) δ 6.90 (s, 1H, NHOH), 6.68 (s, 1H, NHCH), 6.07 (m, 1H, H-3), 6.02–5.98 (m, 1H, H-2), 5.39–5.37 (m, 1H, H-1), 4.39–4.37 (m, 1H, H-4), 3.97–3.86 (m, 2H, H-5, NHOH, OH), 2.00 (br s, 3H, CH_3). ^{13}C NMR (101 MHz, D_2O) δ 130.1 (C-2), 124.9 (C-3), 90.4 (C-1), 70.5 (C-4), 66.0 (C-5), 19.6 (CH_3).

5.4. CA inhibition assays

CO_2 hydrase assay: an Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalyzed CO_2 hydration activity.[13] Phenol red (at a concentration of 0.2 mM) has been used as an indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled–deionized water, and dilutions up to 0.01 nM were done thereafter with distilled–deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, to allow for the formation of the E–I complex. The inhibition constants were obtained by nonlinear least squares methods using the Cheng–Prusoff equation and represent the mean from at least three different determinations. Errors were in the range of ±5–10% of the reported K_I values. CA isoforms were recombinant enzymes obtained in house as reported earlier.[14–16] The enzyme concentrations in the assay system were: hCA I: 13.2 nM; hCA II: 8.4 nM; hCA IX: 7.9 nM; hCA XII: 15.2 nM; hCA XIV: 10.7 nM.

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Supplementary data

Supplementary data (^{1}H NMR and ^{13}C NMR spectra of products) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.09.053.

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