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Spiranic D-*gluco*-configured *N*-substituted thiohydantoins as potential enzymatic inhibitors

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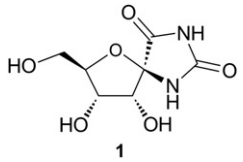
ABSTRACT

We have explored the preparation of conformationally-restricted pseudonucleosides bearing a spiranic thiohydantoin scaffold on C-3 of the sugar moiety by coupling partially protected 3-amino-3-methoxycarbonyl and 3-isothiocyanato-3-methoxycarbonyl glucofuranose derivatives with alkyl(aryl)isothiocyanates or with alkyl(aryl)amines; the key step is a spontaneous or thermal-induced intramolecular nucleophilic substitution of transient thioureas. Upon deprotection, final spiranic thiohydantoins were evaluated as glycosidase and glycogen phosphorylase inhibitors.

1. Introduction

The preparation of nucleoside analogues is a challenging task in Organic and Medicinal Chemistry, due to the important biological activities exhibited by many of such derivatives, considered to be potential therapeutic agents mainly as antiviral and anticancer drugs.[1] Among the plethora of derivatives reported in the literature, deoxy nucleosides,[2] acyclic[3] and carbocyclic[4] nucleosides, thio-,[5] aza-[6] and selenonucleosides,[7] or derivatives bearing modified heterocyclic scaffolds[8] have emerged as new families with enhanced activities and improved bioavailability.[9]

A relatively novel family of nucleosides is comprised of spironucleosides, where carbohydrate derivatives and heterocyclic moieties share a spiranic carbon atom.[10] Such structure has gained attention since the isolation in 1991 of (+)-hydantocidin **1** from the cultured broth of *Streptomyces hygroscopicus*;[11] hydantocidin was found to possess a potent herbicidal activity towards numerous weeds, and to be a plant growth regulator, and at the same time, it lacked toxicity against microorganisms, fishes and mammals.[12]



1

The interesting biological properties exerted by hydantocidin prompted researchers to carry out not only its total synthesis[13] but also to prepare analogues by modifying the sugar moiety,[14] the heterocyclic ring,[15] or by modifying both units.[16] Glycopyranosylidene-spiro derivatives bearing hydantoin,[17] thiohydantoin.[17] or oxathiazole[18] scaffolds were found to be among the most potent inhibitors of glycogen phosphorylase, an enzyme that catalyzes glycogen breakdown, and therefore a potential target in the treatment of metabolic disorder diseases such as diabetes type II.[19] Some other spiranic derivatives structurally related to hydantocidin turned out to be potent salivary α-amylase,[20] or adenylosuccinate synthetase inhibitors.[21]

In this context, we have recently reported a series of spiranic pseudonucleosides bearing oxazolidine-, imidazolidine-2-thione (selone), thiohydantoin-, perhydrooxazine- and azetidine-based heterocycles.[22]

2. Results and discussion

In this communication we have explored the coupling of isothiocyanates and amines with protected 3-amino-3-methoxycarbonyl and with 3-isothiocyanato-3-methoxycarbonyl glucofuranose derivatives **3** and **4**,[22a] respectively, to furnish conformationally-restricted pseudonucleosides **17**–**28** (Scheme 1), where the spiranic annulation takes place at the C-3 of the sugar residue. It has been claimed that reducing the conformational flexibility in nucleosides and nucleotides[23] might enable the optimal puckering between the nucleoside or nucleotide and the

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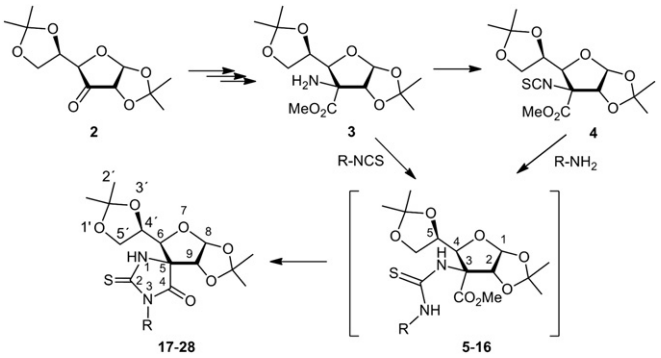


Scheme 1. Preparation of protected 5-spirothiohydantoins **17**–**28**.

interacting biomolecule, due to entropic factors;[24] furthermore, it has also been reported an increased stability of some constrained nucleosides upon radical-induced degradation reactions.[25]

Intermediates **3** and **4** can be accessed starting from 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose **2**,[26] by nucleophilic addition of chloroform to the ketone promoted by LiHMDS (lithium hexamethyldisilazide), followed by modified Corey–Link reaction[27] with NaN_3, MeOH and DBU to furnish, via a transient dichloroepoxy intermediate, the corresponding 3-azido-3-methoxycarbonyl derivative[28] in a complete stereoselective fashion. Reduction of the azido group under catalytic hydrogenation afforded **3**. Conversion of **3** into isothiocyanate derivative **4** was accomplished by treatment with thiophosgene in a heterogeneous $CaCO_3/CH_2Cl_2/H_2O$ system.[22a]

Treatment of aminoester **3** or isothiocyanato ester **4** with an aryl/alkyl isothiocyanate (Table 1, entries 1 and 9) or with an aryl/alkyl amine (Table 1, entries 2–8, 10–12), respectively, resulted in the formation of intermediate thioureas **5**–**16**, which either spontaneously or promoted by heating and/or base underwent an intramolecular nucleophilic substitution on the carbonyl carbon (Scheme 1) to give a thiohydantoin ring with a spiranic annulation (compounds **17**–**28**); such derivatives can be considered as structural analogues of natural (+)-hydantocidin **1**.

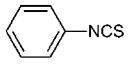
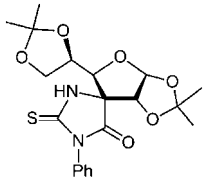
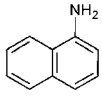
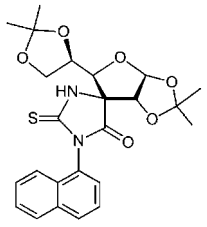
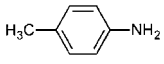
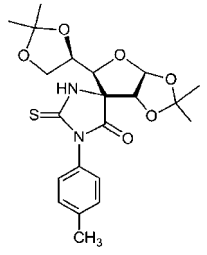
This procedure was successfully applied to the preparation of *N*-substituted thiohydantoins bearing aryl (phenyl, naphthyl, *p*-tolyl) and alkyl residues, including sugar moieties (indolylethyl, *n*-butyl, cyclohexyl, per-*O*-acylated β-D-glucopyranosyl, α-D-glucopyranos-2-yl and α-D-arabinopyranosyl) in moderate to almost quantitative yields (Table 1). When per *O*-acetylated β-D-glucopyranosylamine and glucosamine hydrobromides[29,30] (Table 1, entries 7 and 8, respectively) were used, an equivalent of Et_3N was added to the reaction mixture.

Furthermore, the use of diamines (Table 1, entries 10, 11) or triamines (Table 1, entry 12) afforded dimeric thiohydantoins **26**, **27** and trimeric derivative **28** in 88–97% yields, as examples of potential multivalent ligands. Multivalency has been used to enhance the potency of certain enzymatic inhibitors, especially against receptors bearing multiple recognition sites; this effect is proposed to occur by the increase of the local concentration of ligands, and it has also been used to modulate the pharmacokinetic properties of the inhibitor.[31]

The formation of thiohydantoins **18**–**22**, **26**, and **28** proceeded smoothly at rt, as the corresponding intermediate thioureas underwent spontaneous cyclization to furnish title compounds. On the other hand, for the synthesis of derivatives **17**, **23**–**25**, **27** higher temperatures ranging from 40 °C to 66 °C (see Experimental) were needed in order to favour the intramolecular cyclization in the thiohydantoin ring formation. For thiohydantoin **24**, besides higher temperatures, basic catalysis was proved to be essential for the cyclization step. In some cases, the transient thiourea could be visualized by TLC as a spot with a lower R_f than the final thiohydantoin.

When α-D-arabinopyranosyl isothiocyanate was coupled with aminoester **3**, thiourea **13** (Scheme 1) was isolated as the only compound even when reaction was heated up to 60 °C; the structure was supported by ^1H NMR and ^{13}C NMR spectra, which showed the presence of the methoxycarbonyl group (3.81 and 53.0 ppm), together with two NH peaks in ^1H NMR spectrum (7.97, 7.31 ppm), which thus evidence the existence of a non-spiranic structure. Resonance of the thiocarbonyl group (184.8 ppm, Table 2) was also consistent with the thiourea moiety. Furthermore, the arabinopyranosyl residue was found to be in the $^1C_4$ conformation, as deduced from the

Table 1
Synthesis of protected D-*gluco*-configured 5-spirothiohydantoins

Entry	Amine	Isothiocyanate	Thiohydantoin	Compound	Yield[a] (%)
1	**3**			**17**	68
2		**4**		**18**	50
3		**4**		**19**	80

(*continued on next page*)

Table 1 (*continued*)

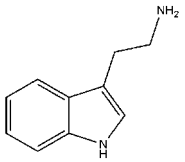
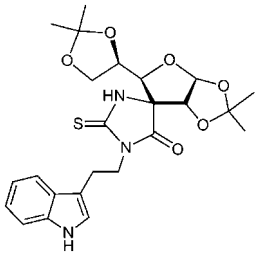
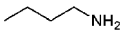
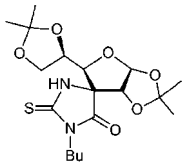
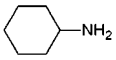
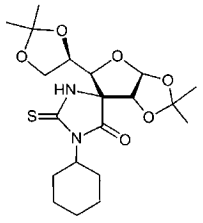
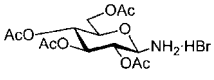
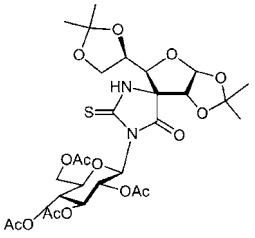
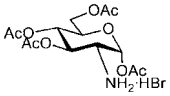
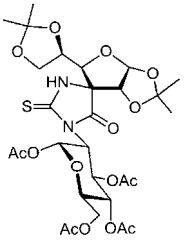
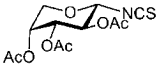
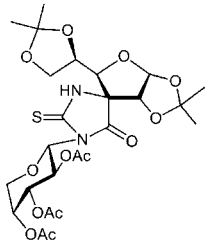
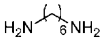
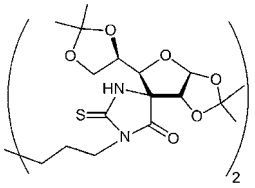
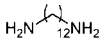
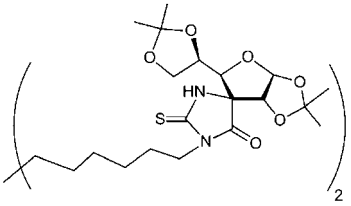
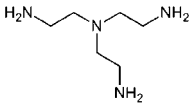
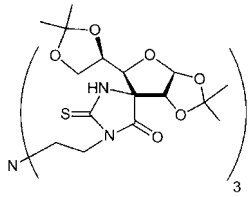
Entry	Amine	Isothiocyanate	Thiohydantoin	Compound	Yield[a] (%)
4		**4**		**20**	79
5		**4**		**21**	88
6		**4**		**22**	94
7		**4**		**23**	76
8		**4**		**24**	23
9	**3**			**25**	93[b]
10		**4**		**26**	93

Table 1 (*continued*)

Entry	Amine	Isothiocyanate	Thiohydantoin	Compound	Yield[a] (%)
11		**4**		**27**	88
12		**4**		**28**	97

[a] Isolated yields.
[b] Starting from intermediate thiourea **13**.

Table 2
Selected NMR data for compounds **3**,**4**,**13**,**17**–**29**

Compd	H-6	H-9	C-2	C-5	C-6	C-9
3[22a,a]	4.67 (H-4)[b]	4.26 (H-2)[b]	—	68.2 (C-3)[b]	82.0 (C-4)[b]	88.7 (C-2)[b]
4[22a,a]	4.60 (H-4)[b]	4.72 (H-2)[b]	142.5 (C=S)[b]	75.0 (C-3)[b]	87.7 (C-4)[b]	82.0 (C-2)[b]
13[a]	4.60 (H-4)[b]	4.82 (H-2′)[b]	184.8 (C=S)[b]	74.2 or 74.3 (C-3′)[b]	80.8 (C-4′)[b]	88.4 (C-2′)[b]
17[a]	4.42	4.77	183.7	71.7	82.9	85.7
18[a]	4.47	4.80	183.9	72.2	81.7	86.5
	4.46[c]	4.91[c]	184.2[c]	72.0[c]	82.7[c]	86.0[c]
19[a]	4.41	4.77	184.0	71.7	82.9	85.7
20[a]	4.42	4.55	183.5	71.1	82.3	85.5
21[a]	4.36	4.63	183.9	71.1	82.3	85.9
22[a]	4.32	4.62	184.2	70.0	82.5	85.8
23[a,d]	4.37	4.62	182.7, 180.6	71.6, 70.4	82.5, 79.5	86.6, 86.2
24[a]	4.39	4.53	183.5	71.0	80.5	88.0
	—[e]	4.67[c]				
25[a]	4.41	4.61	182.7	70.0	82.4	85.6
26[a]	4.35	4.64	183.7	71.1	82.3	85.8
27[a]	4.38–4.35	4.63	183.9	71.1	82.3	85.9
28[a]	4.35	4.70	183.3	71.1	82.2	85.8
29[f]	4.30	4.68	184.5	70.9	83.3	86.8

[a] In $CDCl_3$.
[b] In brackets, sugar numbering.
[c] Minor atropisomer.
[d] A roughly 1:1 mixture of atropisomers.
[e] Not observed.
[f] In CD_3OD.

high $J_{1,2}$, $J_{2,3}$ coupling constants (9.6 Hz), together with the low value for the $J_{3,4}$ coupling constant (3.3 Hz). These data are in accordance with reported data for per-*O*-acylated arabinosyl thioureas.[32] Only when thiourea **13** was refluxed in THF, the corresponding *N*-α-D-arabinopyranosyl-thiohydantoin **25** (Table 1, entry 9) was obtained in excellent yield (93%) after chromatographic purification.

Thiohydantoins **17**–**28** lacked the signal for the methoxycarbonyl group in ^1H and ^{13}C NMR spectra, and only one NH group was observed ranging from 7.26 to 9.58 ppm. Compounds **17**–**28** exhibited a shielding of proton H-6 of roughly 0.2–0.3 ppm when compared with parent α-amino ester **3** and α-isothiocyanato ester **4**,[22a] as depicted in Table 2. Moreover, resonances of spiranic carbon C-5 were deshielded as compared with α-amino ester **3** (ca. 2–4 ppm), and shielded with respect to isothiocyanato ester **4** (ca. 3–5 ppm); these data are in agreement with the observation that an adjacent carbon to an NCS group is deshielded.[22a]

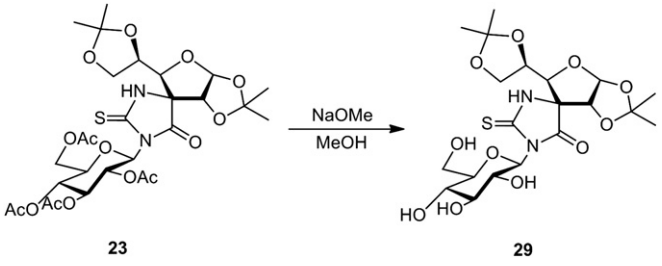
Free rotation around the N–R bond was restricted when bulky substituents were present; thus, for thiohydantoins **18**, **23** and **24**, two families of atropisomeric compounds were detected in their NMR spectra at rt. For derivative **18**, bearing a naphthyl residue, the ratio of the major atropisomer decreased upon heating in DMSO-d_6 from rt to 90 °C from 4.1:1 to 2.9:1, as depicted in Table 3, although no coalescence was reached. The maximum energy level must be found when the aromatic ring is coplanar with the thiohydantoin ring, due to steric hindrance between the naphthyl moiety and the carbonyl and thiocarbonyl groups. On the other hand, the local minima for these two atropisomers must be found when the aromatic system is in a perpendicular orientation with respect to the thiohydantoin ring. *N*-β-D-glucopyranosyl and *N*-α-D-glucopyranos-2-yl thiohydantoins derivatives **23** and **24** also showed

Table 3
Atropisomeric mixtures for compound **18**

Solvent	Temperature	Ratio
$CDCl_3$	rt	3.8:1
DMSO-d_6	rt	4.1:1
DMSO-d_6	60 °C	4.0:1
DMSO-d_6	90 °C	2.9:1

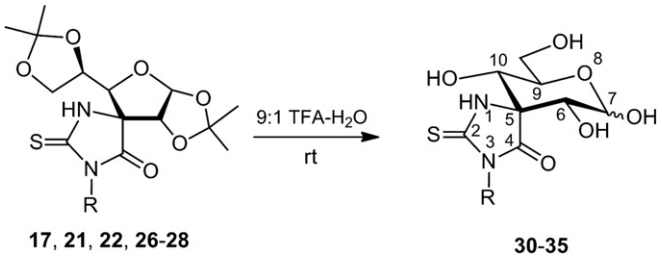
atropisomerism, the rotamers being in a ratio roughly 1:1 and a 6:1, respectively in $CDCl_3$ at rt. In these cases, the presence of a per-*O*-acylated sugar moiety prevented the free rotation around the N–R bond. In thiohydantoin **24**, changing the solvent to DMSO-d_6 led to only one rotamer in the sample.

It is noteworthy that when the glucopyranosyl moiety of **23** was partially deprotected using methanolic NaOMe (Scheme 2), no atropisomerism was observed for the corresponding *O*-deacylated derivative **29**; it is therefore clear that in compound **23** the presence of the acetoxy groups prevents free rotation and thus leads to the presence of two atropisomeric compounds, observable at rt.



Scheme 2. *O*-deacetylation of glucopyranosyl thiohydantoin **23**.

Compounds **17**, **21**, **22**, **26**–**28** were deprotected under acidic conditions (9:1 TFA/H_2O), as depicted in Scheme 3 to afford the corresponding spiranic thiohydantoins **30**–**35** in almost quantitative yields; trimeric thiohydantoin **35** was isolated as its trifluoroacetate salt.



Scheme 3. Deprotection of thiohydantoins.

NMR data of compounds **30**–**35** suggested that they are in the pyranosic form (Table 4) and as solvent-dependent anomeric equilibrium, where the β-anomers are present in a higher ratio (Table 5). The pyranoid structure was evidenced by the high coupling constants $J_{6,7}$ (7.8 Hz-8.1 Hz) and $J_{9,10}$ (9.5–10.2 Hz), thus indicating a trans-arrangement of such protons.

Table 5
Anomeric ratios for thiohydantoins **30**–**35**

Compd	30	31	32	33	34	35
α/β ratio	1:9.9[a] 1:5[b]	1:3.5[b] 1:2.9[c]	1:2.9[b] 1:4[c]	1:10[a]	1:25[b]	1:19[a]

[a] D_2O.
[b] CD_3OD.
[c] DMSO-d_6.

Furthermore, resonances of the adjacent carbons to the thiohydantoin scaffold (C-10 and C-6 for the pyranoid structures) are strongly shielded for derivatives **30**–**35** (Table 4) in comparison with these data for protected furanoid counterparts (C-6, C-9). For example, C-10 showed a resonance in the range 68.7–70.3 ppm for thiohydantoins **30**–**35**, whereas the same carbon (C-6) resonated in the range 83.3–81.7 ppm for the corresponding furanoid derivatives (Table 2). These observations are again indicative of the final compounds exhibiting a pyranoid structure, and are also fulfilled for the corresponding minor α-anomers.

Unprotected thiohydantoins **30**–**33** and **35** were assayed as glycosidase inhibitors (α-glucosidase, β-glucosidase, β-galactosidase) and glycogen phosphorylase b inhibitors (Table 6). None of the tested compound showed inhibition against glycogen phosphorylase. Of the simple thiohydantoins, only compound **30**, bearing an aromatic residue on *N*-3, showed a weak inhibition (8% at 1 mM concentration) against β-glucosidase (almonds) and β-galactosidase (*Aspergillus oryzae*). A similar inhibition was found for dimeric thiohydantoin **33** against α-glucosidase (baker's yeast) and

Table 6
Inhibition activities of compounds **30**–**33**, **35**[a]

Compound	30	31	32	33	35
α-Glucosidase (baker's yeast)	N.I.[b]	N.I.	N.I.	7%	N.I.
β-Glucosidase (almonds)	8% (4.07)	N.I.	N.I.	6%	26% (1.74)
β-Galactosidase (*A. oryzae*)	8%	N.I.	N.I.	N.I.	N.I.
β-Galactosidase (*E. coli*)	N.I.	25%	N.I.	N.I.	25%
Glycogen phosphorylase b (rabbit muscle)	N.I.	N.I.	N.I.	N.I.	N.I.

[a] In brackets K_i values (mM).
[b] No inhibition at 1 mM concentration.

Table 4
Selected NMR data for compounds **30**–**35**

Compd	H-6	H-9	H-10	$J_{6,7}$ (Hz)	C-5	C-9	C-10
30[a]	3.47[b] 3.88[c]	4.17[b] —[c,d]	3.75[b] —[c,d]	8.1[b] 4.7[c]	74.8 or 74.7[b,e] —[c,d]	74.8 or 74.7[b,e] —[c,d]	70.3[b] —[c,d]
31[f]	3.24[b] —[c,d]	3.90[b] 3.91–3.85[c]	3.47[b] —[c,d]	7.8[b] 3.2[c]	72.2[b] 74.1[c]	73.5[b] 67.9[c]	68.2[b] 67.3[c]
32[f]	3.22[b] 3.65–3.02[c]	3.90[b] 3.86[c]	3.45[b] 3.55[c]	8.0[b] 4.0[c]	71.3[b] 73.1[c]	73.4[b] 67.7[c]	67.5[b] 67.5[c]
33[a]	3.56[b] 4.02[c]	4.23[b] 4.26[c]	3.83[b] —[c,d]	8.1[b] 4.5[c]	73.6 or 73.7[b,e] —[c,d]	73.7 or 73.6[b,e] —[c,d]	68.7[b] —[c,d]
34[a]	3.37[b] —[c,d]	4.17[b] 4.13[c,d]	3.51[b] —[c,d]	8.0[b] 4.5[c]	74.3[b] —[c,d]	74.8[b] —[c,d]	70.1[b] —[c,d]
35[g]	3.57[b] 4.00[c]	4.21 [b] —[c,d]	3.84[b] —[c,d]	8.1[b] 4.8[c]	73.0, 73.6[b,e] —[c,d]	73.6 or 73.0[b,e] —[c,d]	68.1[b] —[c,d]

[a] In CD_3OD.
[b] β-anomer.
[c] α-Anomer.
[d] Not observed.
[e] Unambiguous assignment not possible.
[f] In DMSO-d_6.
[g] In D_2O.

β-glucosidase (almonds). Although quite moderate, multivalent thiohydantoin **35**, exhibiting a symmetrical trimeric structure, was found to be a weak inhibitor of β-glucosidase and of β-galactosidase (*Escherichia coli*) (26 and 25%, respectively at 1 mM concentration).

3. Conclusions

We have explored the high-yielding preparation of pseudonucleosides of D-*gluco* configuration and a locked conformation bearing spiro-annulated *N*-substituted thiohydantoins in the C-3 position of the sugar moiety; for that purpose we used an *O*-protected α-amino or α-isotiocyanate ester as the key intermediate, which was coupled with alkyl(aryl) isothiocyanates or amines, including sugar derivatives. This methodology gave access to furanoid spiranic derivatives that were transformed into pyranoid scaffolds upon acidic deprotection. Some unprotected derivatives were found to be weak inhibitors of glycosidases.

4. Experimental section

4.1. General procedures

Optical rotations were measured with a Jasco P-2000 polarimeter. ^{1}H (300 and 500 MHz) and ^{13}C (75.5 and 125.7 MHz) NMR spectra were recorded on Bruker Avance-300 and Avance-500 spectrometers and the indicated spectra data were registered at rt. The assignments of ^{1}H and ^{13}C signals were confirmed by homonuclear COSY and heteronuclear 2D correlated spectra, respectively. Mass spectra (CI and LSI) were recorded on Micromass AutoSpec-Q mass spectrometers with a resolution of 1000 or 10,000 (10% valley definition). For LSI spectra, ions were produced by a beam of xenon atoms and Cs^+ ions, respectively, using thioglycerol as matrix and NaI as additive. TLC was performed on aluminium pre-coated sheets (E. Merck Silica Gel 60 F_{254}); spots were visualized by UV light, by charring with 10% H_2SO_4 in EtOH or with 3% ninhydrin in EtOH. Column chromatography was performed using E. Merck Silica Gel 60 (40–63 μm). Compound **2** was prepared according to the described literature procedure.[26] Compounds **3** and **4** were prepared as we described in a previous work.[22a]

*4.1.1. (5S,6S,8R,9R,4′R)-6-(2′,2′-Dimethyl-1′,3′-dioxolan-4′-yl)-8,9-(dimethylmethylenedioxy)-3-phenyl-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**17**).* To a solution of aminoester **3** (63.8 mg, 0.20 mmol) in THF (1.5 mL) was added phenyl isothiocyanate (36 μL, 0.30 mmol, 1.5 equiv). The corresponding solution was stirred at rt for 24 h and at 60 °C for 16 h. Then, the solvent was removed under reduced pressure and the residue was purified by column chromatography (7:3 hexane/EtOAc) to give **17** as an amorphous solid (91 mg, 68%). $[\alpha]_D^{23}$ +60 (*c* 1.21, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.58 (s, 1H, NH), 7.52–7.45 (m, 3H, Ar), 7.33–7.31 (m, 2H, Ar), 5.95 (d, 1H, $J_{8,9}$=3.6 Hz, H-8), 4.77 (d, 1H, H-9), 4.42 (d, 1H, $J_{6,4'}$=9.3 Hz, H-6), 4.22 (ddd, 1H, $J_{4',5'a}$=5.7 Hz, $J_{4',5'b}$=3.6 Hz, H-4′), 4.12 (dd, 1H, $J_{5'a,5'b}$=9.0 Hz, H-5′a), 4.02 (dd, 1H, H-5′b), 1.64 (s, 3H, CH_3), 1.33 (s, 6H, $2CH_3$), 1.30 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 183.7 (C-2), 167.9 (C-4), 132.9, 129.4, 129.2, 128.3 (Ar), 114.6, 110.4 ($2CMe_2$), 105.8 (C-8), 85.7 (C-9), 82.9 (C-6), 73.4 (C-4′), 71.7 (C-5), 67.4 (C-5′), 26.9, 26.5, 25.4 ($4CH_3$); HRLSI-MS *m*/*z* calcd for $C_{20}H_{24}N_2NaO_6S$, $[M+Na]^+$: 443.1253, found: 443.1227.

4.2. General procedures for the preparation of thiohydantoins 18–24, 26–28

Method A: To a solution of isothiocyanate **4** (100 mg, 0.28 mmol) in THF (1.5 mL) was added the corresponding amine (0.42 mmol for thiohydantoins **18**–**22**, 0.14 mmol for dimeric thiohydantoins **26** and **27** and 0.046 mmol for trimeric thiohydantoin **28**) and the solution was kept stirring at *x* °C for *y* h, until TLC showed disappearance of the starting material. The solvent was then removed under reduced pressure and the residue was purified by column chromatography using the eluant indicated in each case.

Method B: To a solution of isothiocyanate **4** (100 mg, 0.28 mmol) in THF (1.5 mL) was added the corresponding amine (0.42 mmol) and triethylamine (0.46 mmol). The solution was kept stirring at *x* °C for *y* h, until TLC showed disappearance of the starting material. The solvent was then removed under reduced pressure and the residue was purified by column chromatography using the eluant indicated in each case.

*4.2.1. (5S,6S,8R,9R,4′R)-6-(2′,2′-Dimethyl-1′,3′-dioxolan-4′-yl)-8,9-(dimethylmethylenedioxy)-3-α-naphthyl-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**18**).* Method A: *x*=25 °C, *y*=24 h. Column chromatography: 50:1 CH_2Cl_2/MeOH. Amorphous solid (65.8 mg, 50%). $[\alpha]_D^{26}$ +39 (*c* 1.15, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): major atropisomer δ 9.37 (s, 1H, NH), 8.03–7.99 (m, 2H, Ar), 7.94 (d, 1H, $J_{H,H}$=7.8 Hz, Ar), 7.60–7.46 (m, 4H, Ar), 5.93 (d, 1H, $J_{8,9}$=3.5 Hz, H-8), 4.80 (d, 1H, H-9), 4.47 (d, 1H, $J_{6,4'}$=9.5 Hz, H-6), 4.38 (ddd, 1H, $J_{4',5'a}$=5.5 Hz, $J_{4',5'b}$=3.0 Hz, H-4′), 4.15–4.09 (m, 2H, H-5′a, H-5′b), 1.63, 1.45, 1.34, 1.21 (4s, 3H each, $4CH_3$); minor atropisomer: δ 9.32 (s, 1H, NH), 7.68 (d, 1H, $J_{H,H}$=8.0 Hz, Ar), 7.41 (d, 1H, $J_{H,H}$=7.2 Hz, Ar), 5.95 (d, 1H, $J_{8,9}$=3.6 Hz, H-8), 4.91 (d, 1H, H-9), 4.46 (d, 1H, $J_{6,4'}$=9.5 Hz, H-6), 4.29 (ddd, 1H, $J_{4',5'a}$=5.7 Hz, $J_{4',5'b}$=3.4 Hz, H-4′), 4.15–4.12 (m, 1H, H-5′a), 4.04 (dd, 1H, $J_{5'a,5'b}$=9.3 Hz, H-5′b), 1.61, 1.40, 1.35, 1.26 (4s, 3H each, $4CH_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$): major atropisomer δ 183.9 (C-2), 168.0 (C-4), 134.5, 130.7, 130.6, 129.5, 128.6, 127.8, 126.9, 126.8, 125.5, 123.6 (Ar), 114.8, 111.0 ($2CMe_2$), 105.3 (C-8), 86.5 (C-9), 81.7 (C-6), 73.3 (C-4′), 72.2 (C-5), 67.2 (C-5′), 27.0, 26.7, 26.6, 25.7 ($4CH_3$); minor atropisomer: δ 184.2 (C-2), 167.9 (C-4), 134.4, 130.5, 130.2, 129.9, 128.8, 127.5, 127.3, 125.4, 122.2 (Ar), 114.8, 110.6 (CMe_2), 105.8 (C-8), 86.0 (C-9), 82.7 (C-6), 73.5 (C-4′), 72.0 (C-5), 67.4 (C-5′), 27.0, 26.7 26.6, 25.6 ($4CH_3$); HRLSI-MS *m*/*z* calcd for $C_{24}H_{26}N_2NaO_6S$, $[M+Na]^+$: 493.1409, found: 493.1418.

*4.2.2. (5S,6S,8R,9R,4′R)-6-(2′,2′-Dimethyl-1′,3′-dioxolan-4′yl)-8,9-(dimethylmetilenedioxy)-2-thioxo-3-p-tolyl-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**19**).* Method A: *x*=25 °C, *y*=24 h. Column chromatography: 50:1 CH_2Cl_2/MeOH. Amorphous solid (97 mg, 80%). $[\alpha]_D^{23}$ +65 (*c* 1.15, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.34 (s, 1H, NH), 7.30–7.29, 7.20–7.18 (m, 4H, Ar), 5.96 (d, 1H, $J_{8,9}$=3.5 Hz, H-8), 4.77 (d, 1H, H-9), 4.41 (d, 1H, $J_{6,4'}$=9.5 Hz, H-6), 4.22 (ddd, 1H, $J_{4',5'a}$=6.0 Hz, $J_{4',5'b}$=4.0 Hz, H-4′), 4.12 (dd, 1H, $J_{5'a,5'b}$=9.0 Hz, H-5′a), 4.01 (dd, 1H, H-5′b), 2.40 (s, 3H, $PhCH_3$), 1.63, 1.33, 1.32, 1.29 (4s, 3H each, $4CH_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 184.0 (C-2), 167.9 (C-4), 139.6, 130.4, 129.9, 128.1 (Ar), 114.7, 110.5 ($2CMe_2$), 105.8 (C-8), 85.7 (C-9), 82.9 (C-6), 73.5 (C-4′), 71.7 (C-5), 67.5 (C-5′), 26.9, 26.6, 25.4 ($4CH_3$), 21.4 (CH_3Ar); HRLSI-MS *m*/*z* calcd for $C_{21}H_{26}N_2NaO_6S$, $[M+Na]^+$: 457.1409, found: 457.1431.

*4.2.3. (5S,6S,8R,9R,4′R)-6-(2′,2′-Dimethyl-1′,3′-dioxolan-4′-yl)-8,9-(dimethylmethylenedioxy)-3-[2‴-(1‴H-indol-3‴-yl)ethyl]-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**20**).* Method A: *x*=25 °C, *y*=24 h. Column chromatography: 99:1 CH_2Cl_2/MeOH. Amorphous solid (107.8 mg, 79%). $[\alpha]_D^{23}$ +70 (*c* 1.08, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.81 (s, 1H, NH), 8.10 (s, 1H, NH), 7.84 (d, 1H, $J_{H,H}$=7.5 Hz, Ar), 7.36 (d, 1H, $J_{H,H}$=8.1 Hz, Ar), 7.25–7.11(m, 3H, Ar), 5.98 (d, 1H, $J_{8,9}$=3.6 Hz, H-8), 4.55 (d, 1H, H-9), 4.42 (m, 1H, H-6), 4.17–3.99 (m, 5H, H-4′, H-5′a, H-5′b, H-1″), 3.13 (m, 2H, H-2″), 1.67, 1.35, 1.34, 1.23 (4s, 3H each, $4CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 183.5 (C-2), 168.4 (C-4), 136.2, 127.4, 122.3, 122.2, 119.6, 119.1 (Ar), 114.6 (CMe_2), 112.2, 111.2 (Ar) 110.3 (CMe_2), 105.5 (C-8), 85.5 (C-9), 82.3 (C-6), 73.2 (C-4′), 71.1 (C-5), 67.3 (C-5′), 42.2 (C-1″), 26.7, 26.6, 26.4, 25.2 ($4CH_3$),

23.1 (C-2″); HRLSI-MS *m*/*z* calcd for $C_{24}H_{29}N_3NaO_6S$, $[M+Na]^+$: 510.1675, found: 510.1684.

*4.2.4. (5S,6S,8R,9R,4′R)-3-Butyl-6-(2′,2′-dimethyl-1′,3′-dioxolan-4′-yl)-8,9-(dimethylmethylenedioxy)-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**21**).* Method A: *x*=25 °C, *y*=1 h. Column chromatography: 4:1→7:3 hexane/EtOAc gradient. Amorphous solid (93 mg, 84%). $[\alpha]_D^{23}$ +44 (*c* 1.34, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.05 (m, 1H, NH), 5.98 (d, 1H, $J_{1,2}$=3.5 Hz, H-8), 4.63 (d, 1H, H-9), 4.36 (d, 1H, $J_{6,4'}$=9.0 Hz, H-6), 4.11 (ddd, 1H, $J_{4',5a}$=6.0 Hz, $J_{4',5b}$=3.5 Hz, H-4′) 4.08 (dd, 1H, $J_{5a',5b'}$=8.5 Hz, H-5′a), 3.98 (dd, 1H, H-5′b), 3.77 (m, 2H, H-1″), 1.68–1.59 (m, 2H, H-2″), 1.65 (s, 3H, CH_3), 1.38 (m, 2H, H-3″), 1.32, 1.31, 1.22 (3s, 3H each, $3CH_3$), 0.94 (t, 3H, $J_{3',4'}$=7.5 Hz, H-4″); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 183.9 (C-2), 168.5 (C-4), 114.6, 110.4 ($2CMe_2$), 105.7 (C-8), 85.9 (C-9), 82.3 (C-6), 73.3 (C-4′), 71.1 (C-5), 67.4 (C-5′), 41.6 (C-1″), 29.5 (C-2″), 26.9, 26.6, 26.5, 25.3 ($4CH_3$), 20.0 (C-3″), 13.8 (C-4″); HRLSI-MS *m*/*z* calcd for $C_{18}H_{28}N_2NaO_6S$, $[M+Na]^+$: 423.1566, found: 423.1583.

*4.2.5. (5S,6S,8R,9R,4′R)-3-Cyclohexyl-6-(2′,2′-dimethyl-1′,3′-dioxolan-4′-yl)-8,9-(dimethylmethylenedioxy)-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**22**).* Method A: *x*=25 °C, *y*=30 min. Column chromatography: 80:1 CH_2Cl_2/MeOH. Amorphous solid (112 mg, 94%). $[\alpha]_D^{23}$ +54 (*c* 0.94, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.26 (s, 1H, NH), 5.96 (d, 1H, $J_{8,9}$=4.0 Hz, H-8), 4.62 (d, 1H, H-9), 4. 44 (tt, 1H, $J_{HH'}$=12.0 Hz, $J_{HH'}$=4.0 Hz, CH cyclohexyl), 4.32 (m, 1H, H-6), 4.12–4.07 (m, 2H, H-4′, H-5′a), 3.96 (m, 1H, H-5′b), 2.31 (dq, 1H, J_{HH}=12.5 Hz, J_{HH}=4.0 Hz, CH_A), 2.16 (dq, 1H, J_{HH}=12.2 Hz, J_{HH}=3.5 Hz, CH_A), 1.83 (m, 2H, $2CH_A$) 1.73–1.67 (m, 2H, $2CH_B$), 1.70–1.64 (m, 1H, CH_A), 1.64, 1.33, 1.32, 1.23 (4s, 3H each, $4CH_3$), 1.36–1.28 (m, 2H, $2CH_B$), 1.21–1.16 (m, 1H, CH_B); ^{13}C NMR (127.5 MHz, $CDCl_3$): δ 184.2 (C-2), 168.3 (C-4), 114.7, 110.4 ($2CMe_2$), 105.6 (C-8), 85.8 (C-9), 82.5 (C-6), 73.3 (C-4′), 70.0 (C-5), 67.4 (C-5′), 55.8 (CH cyclohexyl), 28.8, 28.3 ($2CH_2$), 26.9, 26.7, 26.6 ($3CH_3$), 26.1, 26.0 ($2CH_2$), 25.4 (CH_3), 25.2 (CH_2); HRLSI-MS *m*/*z* calcd for $C_{20}H_{30}N_2NaO_6S$, $[M+Na]^+$: 449.1722, found: 449.1718.

*4.2.6. (5S,6S,8R,9R,4′R)-6-(2′,2′-Dimethyl-1′,3′-dioxolan-4′-yl)-8,9-(dimethylmethylenedioxy)-3-(2″,3″,4″,6″-tetra-O-acetyl-β-D-glucopyranosyl)-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**23**).* Method B: *x*=40 °C, *y*=12 h. Column chromatography: 3:2 Et_2O/hexane. Amorphous solid (142 mg, 76%). $[\alpha]_D^{23}$ +9 (*c* 1.17, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): roughly 1:1* atropisomeric mixture: δ 6.43 (m, 1H, H-2″), 6.04–6.95 (m, 3H, H-8, H-8*, H-1″), 5.82 (m, 1H, H-2″*), 5.46 (m, 1H, H-1″*), 5.29 (t, 2H, $J_{2'',3''}$=$J_{3'',4''}$=9.5 Hz, H-3″, H-3″*), 5.22 (t, 2H, $J_{4'',5''}$=9.5 Hz, H-4″, H-4″*), 4.62 (m, 2H, H-9, H-9*), 4.37 (d, 2H, $J_{6,4'}$=8.5 Hz, H-6, H-6*), 4.31 (br d, 2H, $J_{6''a,6''b}$=12.0 Hz, H-6″a, H-6″a*), 4.12 (dd, 2H, $J_{5'',6''b}$=5.0 Hz, H-6″b, H-6″b*), 4.14–4.11 (m, 4H, H-4′, H-4′*, H-5′a, H-5′a*), 4.01 (m, 2H, H-5′b, H-5′b*), 3.81 (ddd, 2H, $J_{5'',6''a}$=2.5 Hz, H-5″, H-5″*), 2.06, 2.05, 2.00, 1.95 (4s, 6H each, 4AcO, 4AcO*), 1.62 (s, 6H, CH_3, CH_3*), 1.35–1.29 (m, 12H, $2CH_3$, $2CH_3$*), 1.25 (s, 6H, CH_3, CH_3*); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 182.7, 180.6 (C-2, C-2*), 170.7 (×2), 170.2, 170.0 (×3), 169.5, 169.2, 168.3, 165.9 (C-4, C-4*, 4C=O, 4C=O*), 114.9, 114.3, 111.2, 110.4 ($2CMe_2$, $2CMe_2$*), 106.3, 104.7 (C-8, C-8*), 86.6, 86.2 (C-9, C-9*), 82.5 (C-6), 81.6 (C-1″), 79.7 (C-1″*), 79.5 (C-6*), 74.3 (C-5″), 73.5 (C-5″*), 72.8 (C-3″, C-3″*, C-4′, C-4′*), 71.6, 70.4 (C-5, C-5*), 68.6, 68.4 (C-2″, C-2″*), 68.1, 67.5 (C-4″, C-4″*), 66.4 (C-5′, C-5′*), 62.0 (C-6″, C-6″*), 26.9 (×2), 26.6 (×3), 26.1, 25.1, 25.0 ($4CH_3$, $4CH_3$*); 20.9, 20.7 ($4COCH_3$, $4COCH_3$*); HRLSI-MS *m*/*z* calcd for $C_{28}H_{38}N_2NaO_{15}S$, $[M+Na]^+$: 697.1891, found: 697.1893.

*4.2.7. (5S,6S,8R,9R,4′R)-6-(2′,2′-dimethyl-1′,3′-dioxolan-4′-yl)-8,9-(dimethylmethylenedioxy)- 3-(1″,3″,4″,6″-tetra-O-acetyl-2″-deoxy-β-d-glucopyranos-2″-yl)-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**24**).* Method B: *x*=40 °C, *y*=12 h. Then, more Et_3N (0.27 mmol) was added and the solution was heated at 60 °C for 12 h. Column chromatography: 4:1 Et_2O/hexane. Amorphous solid (74 mg, 23%). $[\alpha]_D^{24}$ +238 (*c* 0.48, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) 6:1 atropisomeric mixture: major atropisomer: δ 6.47 (dd, 1H, $J_{2'',3''}$=12.0 Hz, $J_{3'',4''}$=9.0 Hz, H-3″), 6.38 (d, 1H, $J_{1'',2''}$=3.0 Hz, H-1″), 5.98 (d, 1H, $J_{8,9}$=4.0 Hz, H-8), 5.10 (dd, 1H, $J_{4'',5''}$=10.0 Hz, H-4″), 5.04 (dd, 1H, H-2″), 4.53 (d, 1H, H-9), 4.39 (d, 1H, $J_{6,4'}$=7.0 Hz, H-6), 4.32 (dd, 1H, $J_{5'',6''a}$=4.0 Hz, $J_{6''a,6''b}$=11.0 Hz, H-6″a), 4.20 (ddd, 1H, $J_{4'',5''}$=10.0 Hz, $J_{5'',6''b}$=2.0 Hz, H-5″), 4.12 (m, 1H, H-4′), 4.10 (dd, 1H, H-6″b), 4.06 (ddd, 1H, $J_{5'a,4'}$=6.4 Hz, $J_{5a',5b'}$=8.8 Hz, H-5′a), 3.85 (dd, 1H, $J_{4',5'b}$=5.5, H-5′b), 2.11, 2.09, 2.02, 1.93 (4s, 3H each, $4COCH_3$), 1.58, 1.32, 1.28, 1.26 (4s, 3H each, $4CH_3$); minor atropisomer: δ 6.23 (d, 1H, $J_{1'',2''}$=3.2 Hz, H-1″), 5.91 (d, 1H, $J_{8,9}$=3.3 Hz, H-8), 4.78 (dd, 1H, $J_{2'',3''}$=11.8 Hz, H-2″), 4.67 (d, 1H, H-9); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.2 (br s, 1H, NH), 6.26 (dd, 1H, $J_{2'',3''}$=11.8 Hz, $J_{3'',4''}$=9.2 Hz, H-3″), 6.12 (d, 1H, $J_{1'',2''}$=3.0 Hz, H-1″), 5.96 (d, 1H, $J_{8,9'}$=3.8 Hz, H-8), 4.98 (t, 1H, $J_{4'',5''}$=9.5 Hz, H-4″), 4.91 (dd, 1H, H-2″), 4.60 (d, 1H, H-9), 4.29–4.22 (m, 3H, H-5, H-5″, H-6a″), 4.05–3.95 (m, 3H, H-4′, H-6b″, H-5a′), 3.84–3.80 (m, 1H, H-5b′), 2.09, 2.02, 1.95, 1.86 (4s, 3H each, $4OCH_3$), 1.42, 1.23, 1.20, 1.17 (4s, 3H each, $4CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) major atropisomer: δ 183.5 (C-2), 170.9, 170.1, 169.5, 169.0, 166.8 (C-4, 4C=O), 114.6, 110.3 ($2CMe_2$), 104.8 (C-8), 89.9 (C-1″), 88.0 (C-9), 80.5 (C-6), 73.0 (C-4′), 71.0 (C-5), 70.1 (C-5″), 69.8 (C-4″), 66.8 (C-5′), 66.4 (C-3″), 61.8 (C-6″), 57.0 (C-2″), 27.3, 26.4, 26.2, 25.1 ($4CH_3$), 21.5, 20.9, 20.8, 20.7 ($4OCH_3$); minor atropisomer: δ 104.9 (C-8), 91.2 (C-1″), 86.3 (C-1), 70.3 (C-5″), 66.9 (C-4″), 66.8 (C-5′), 55.2 (C-2″); HRLSI-MS *m*/*z* calcd for $C_{28}H_{38}N_2NaO_{15}S$, $[M+Na]^+$: 697.1891, found: 697.1917.

*4.2.8. (5′S,6′S,8′R,9′R,4″R)-1,6-Bis[6′-(2″,2″-dimethyl-1″,3″-dioxolan-4″-yl)-8′,9′-(dimethylmethylenedioxy)-4′-oxo-2′-thioxo-7′-oxa-1′,3′-diazaspiro[4.4]nonan-3′-yl]hexane (**26**).* Method A: *x*=25 °C, *y*=2 h. Column chromatography: 40:1 CH_2Cl_2/MeOH. Amorphous solid (101 mg, 93%). $[\alpha]_D^{23}$ +55 (*c* 0.72, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.97 (d, 1H, $J_{8',9'}$=3.6 Hz, H-8′), 5.29 (s, 1H, NH), 4.64 (d, 1H, H-9′), 4.35 (d, 1H, $J_{6',4''}$=8.6 Hz, H-6′), 4.09 (m, 2H, H-4″, H-5″a), 3.97 (m, 1H, H-5″b), 3.78–3.71 (m, 2H, H-1), 1.68–1.64 (m, 2H, H-2), 1.65 (s, 3H, CH_3), 1.40 (m, 2H, H-3), 1.33, 1.31, 1.23 (3s, 3H each, $3CH_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 183.7 (C-2′), 168.4 (C-4′), 114.6, 110.4 ($2CMe_2$), 105.6 (C-8′), 85.8 (C-9′), 82.3 (C-6′), 73.3 (C-4″), 71.1 (C-5′), 67.4 (C-5″), 41.6 (C-1), 27.3, 26.9, 26.6, 26.5, 26.4, 25.4 (C-2, C-3, 4 CH_3); HRLSI-MS *m*/*z* calcd for $C_{34}H_{50}N_4NaO_{12}S_2$, $[M+Na]^+$: 793.2754, found: 793.2746.

*4.2.9. (5′S,6′S,8′R,9′R,4″R)-1,12-Bis[6′-(2″,2″-dimethyl-1″,3″-dioxolan-4″-yl)-8′,9′-(dimethylmethylenedioxy)-4′-oxo-2′-thioxo-7′-oxa-1′,3′-diazaspiro[4.4]nonan-3′-yl]dodecane (**27**).* Method A: *x*=25 °C, *y*=16 h, then, *x*=40 °C, *y*=1 h. Column chromatography: 50:1 CH_2Cl_2/MeOH. Amorphous solid (105 mg, 88%). $[\alpha]_D^{24}$ +35 (*c* 1.27, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.68 (s, 1H, NH), 5.97 (d, 1H, $J_{8',9'}$=4.0 Hz, H-8′), 4.63 (d, 1H, H-9′), 4.38–4.35 (m, 1H, H-6′), 4.12–4.07 (m, 2H, H-4″, H-5″a), 4.00–3.96 (m, 1H, H-5″b), 3.76 (m, 2H, H-1), 1.68–1.60 (m, 2H, H-2), 1.65 (s, 3H, CH_3), 1.36–1.22 (m, 8H, H-3–H-6), 1.33, 1.31, 1.23 (3s, 3H each, $3CH_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 183.9 (C-2′), 168.4 (C-4′), 114.7, 110.4 ($2CMe_2$), 105.6 (C-8′), 85.9 (C-9′), 82.3 (C-6′), 73.4 (C-4″), 71.1 (C-5′), 67.4 (C-5″), 41.9 (C-1), 29.7, 29.6, 29.3 ($3CH_2$), 27.4, 26.9, 26.8, 26.6, 26.5 ($2CH_2$, $3CH_3$), 25.3 (CH_3); HRLSI-MS *m*/*z* calcd for $C_{40}H_{62}N_4NaO_{12}S_2$, $[M+Na]^+$: 877.3703, found: 877.3712.

*4.2.10. (5′S,6′S,8′R,9′R,4″R)-Tris(2-[6′-(2″,2″-dimethyl-1″,3″-dioxolan-4″-yl)-8′,9′-(dimethylmethylenedioxy)-4′-oxo-2′-thioxo-7′-oxa-1′,3′-diazaspiro[4.4]nonan-3′-yl]ethyl)amine (**28**).* Method A: *x*=25 °C, *y*=24 h. Column chromatography: 20:1 CH_2Cl_2/MeOH. Amorphous

solid (51 mg, 97%). $[\alpha]_D^{23}$ +50 (*c* 1.03, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 8.62 (m, 1H, NH), 5.98 (d, 1H, $J_{8'-9'}$=3.6 Hz, H-8′), 4.70 (d, 1H, H-9′), 4.35 (d, 1H, $J_{6',4''}$=8.1 Hz, H-6′), 4.11–4.07 (m, 2H, H-4″, H-5″a), 4.00–3.84 (m, 3H, H-5″b, CH_2), 2.86 (m, 2H, CH_2), 1.63 (s, 3H, CH_3), 1.33 (s, 6H, $2CH_3$), 1.25 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 183.3 (C-2′), 168.2 (C-4′), 114.6, 110.4 ($2CMe_2$), 105.6 (C-8′), 85.8 (C-9′), 82.2 (C-6′), 73.3 (C-4″), 71.1 (C-5′), 67.3 (C-5″), 50.6 (C-1) 39.7 (C-2), 26.9, 26.6, 26.5, 25.2 ($4CH_3$); HRLSI-MS *m*/*z* calcd for $C_{48}H_{69}N_7NaO_{18}S_3$, $[M+Na]^+$: 1150.3759, found: 1150.3719.

*4.2.11. N-(2,3,4-Tri-O-acetyl-α-D-arabinopyranosyl)-N′-(3S-3-deoxy-1,2:5,6-di-O-isopropylidene-3-methoxycarbonyl-α-D-ribo-hexafuranos-3-yl)thiourea (**13**).* To a solution of aminoester **3** (61 mg, 0.19 mmol) in THF (2 mL) was added commercially available 2,3,4-tri-*O*-acetyl-α-D-arabinopyranosyl isothiocyanate (66 mg, 0.21 mmol, 1.10 equiv) and the reaction was kept stirring at 40 °C for 3.5 h and at 60 °C for 6 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (9:1 hexane/$Et_2O \rightarrow Et_2O$) to afford **13** as an amorphous solid (160.2 mg, 81%). $[\alpha]_D^{23}$ 0 (*c* 1.09, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, 1H, $J_{NH,1}$=6.6, NH), 7.31 (s, 1H, NH'), 6.03 (d, 1H, $J_{1',2'}$=3.9 Hz, H-1′), 5.64 (t, 1H, $J_{1,2}$=9.6 Hz, H-1); 5.33 (m, 1H, H-4), 5.20 (t, 1H, $J_{2,3}$=9.6 Hz, H-2), 5.12 (dd, 1H, $J_{3,4}$=3.3 Hz, H-3), 4.82 (d, 1H, H-2′), 4.60 (d, 1H, $J_{4',5'}$=8.1 Hz, H-4′), 4.23 (m, 1H, H-5′), 4.12 (dd, 1H, $J_{5',6a'}$=6.3 Hz, $J_{6a',6b'}$=9.0 Hz, H-6a′), 4.01 (dd, 1H, $J_{5',6b'}$=4.5 Hz, H-6b′), 3.96 (dd, 1H, $J_{4,5a}$=1.5 Hz, $J_{5a\text{-}5b}$=12.9 Hz, H-5a); 3.81 (s, 3H, OMe), 3.76 (d, 1H, H-5b); 2.10 (×2), 2.0 (3s, 3H each, 3AcO), 1.53, 1.51, 1.44, 1.29 (3s, 3H each, $3CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 184.8 (C=S), 170.7, 170.2, 169.9, 166.2 (4C=O), 113.3, 110.9 (2×CMe_2), 104.1 (C-1′), 88.4 (C-2′), 84.6 (C-1), 80.8 (C-4′), 72.4, 72.3 (C-3′, C-5′), 71.3 (C-3), 68.5 (C-4), 67.8 (C-2), 67.2 (C-6′), 66.2 (C-5), 53.0 (OMe), 26.8, 26.2 (×2), 25.2 (4×CH_3), 21.0, 20.7 (3COMe); HRLSI-MS *m*/*z* calcd for $C_{26}H_{38}N_2NaO_{14}S$, $[M+Na]^+$: 657.1941, found: 657.1934.

*4.2.12. (5S,6S,8R,9R,4′R)-6-(2′,2′-Dimethyl-1′,3′-dioxolan-4′-yl)-8,9-(dimethylmethylenedioxy)-2-thioxo-3-(2″,3″,4″-tri-O-acetyl-β-d-arabinopyranosyl)-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**25**).* A solution of tiourea **13** (160.2 mg, 0.25 mmol) in THF (2 mL) was refluxed for 2 h; then the solvent was removed under reduced pressure and the residue was purified by column chromatography (60:1 CH_2Cl_2/MeOH) to give **25** as an amorphous solid (141.5 mg, 93%). $[\alpha]_D^{23}$ +30 (*c* 1.11, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 8.21 (s, 1H, NH), 6.14 (br t, 1H, $J_{1'',2''}$=$J_{2'',3''}$=9, H-2″), 5.93 (d, 1H, $J_{8,9}$=3.6 Hz, H-8), 5.68 (d, 1H, H-1″), 5.25 (m, 1H, H-4″), 5.16 (dd, 1H, $J_{3''-4''}$=3.3 Hz, H-3″), 4.61 (d, 1H, H-9), 4.41 (d, 1H, $J_{6,4'}$=8.4 Hz, H-6), 4.10–4.01 (m, 3H, H-4′, H-5′a, H-5″a), 3.98–3.94 (m, 1H, H-5′b), 3.73 (d, 1H, $J_{5''a,5''b}$=12.9, H-5″b), 2.17, 2.00, 1.99 (3s, 3H each, 3AcO); 1.67, 1.38, 1.30, 1.21 (4s, 3H each, $4CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 182.7 (C-2); 170.9, 170.2 (×2), 166.4 (C-4, 3C=O); 114.9, 110.6 ($2CMe_2$), 105.5 (C-8), 85.6 (C-9), 82.4 (C-6, C-1″), 73.4 (C-4′), 71.3 (C-3″), 70.0 (C-5), 68.1 (C-4″), 67.3 (C-5′), 66.7 (C-2″), 66.1 (C-5″), 27.0, 26.8, 25.7, 25.1 ($4CH_3$); 21.1, 20.8 (×2) ($3COCH_3$); HRLSI-MS *m*/*z* calcd for $C_{25}H_{34}N_2NaO_{13}S$, $[M+Na]^+$: 625.1679, found: 625.1695.

*4.2.13. (5S,6S,8R,9R,4′R)-6-(2′,2′-Dimethyl-1′,3′-dioxolan-4′-yl)-8,9-(dimethylmethylenedioxy)-3-β-D-glucopyranosyl-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**29**).* To a solution of thiohydantoin **23** (71 mg, 0.11 mmol) in anhydrous MeOH (4 mL) was added NaOMe (24 mg, 0.44 mmol) and the corresponding mixture was stirred at rt for 1.5 h. Then it was neutralized with Amberlite IR-120(H^+) resin and filtrated. The filtrate was concentrated to dryness and the residue was purified by column chromatography (40:1 →10:1 CH_2Cl_2/MeOH) to give **29** as an amorphous solid (29.5 mg, 56%). $[\alpha]_D^{23}$ +56 (*c* 0.85, MeOH); ^1H NMR (300 MHz, CD_3OD): δ 5.98 (d, 1H, $J_{8,9}$=3.6 Hz, H-8), 5.69 (d, 1H, $J_{1'',2''}$=9.3 Hz, H-1″), 4.68 (d, 1H, H-9), 4.38 (br t, 1H, $J_{2'',3''}$=8.5 Hz, H-2″), 4.30 (d, 1H, $J_{6,4'}$=8.8, H-6), 4.13 (m, 1H, H-4′), 4.10 (dd, 1H, $J_{4',5'a}$=5.7 Hz, $J_{5a',5'b}$=11.4 Hz, H-5′a), 4.02 (m, 1H, H-5′b), 3.88 (d, 1H, $J_{5'',6''a}$=12.3 Hz, H-6″a), 3.69 (m, 1H, H-6″b), 3.46–3.41 (m, 3H, H-3″, H-4″, H-5″), 1.58, 1.44, 1.33, 1.28 (4s, 3H each, $4CH_3$); ^{13}C NMR (75.5 MHz, CD_3OD): δ 184.5 (C-2), 169.9 (C-4), 115.4, 112.1 ($2CCH_3$); 107.0 (C-8), 86.8 (C-9), 85.5 (C-1″), 83.3 (C-6), 81.2, 78.7 (C-3″, C-5″), 74.3 (C-4′), 71.2 (C-4″), 70.9 (C-5), 69.9 (C-2″), 67.6 (C-5′), 63.3 (C-6″), 26.9 (×2), 26.7, 25.2 ($4CH_3$); HRCI-MS *m*/*z* calcd for $C_{20}H_{31}N_2O_{11}S$, $[M+H]^+$: 507.1649, found: 507.1641.

4.3. General procedure for the deprotection of thiohydantoins

A solution of the protected thiohydantoins (*x* mg) in 9:1 TFA–H_2O (y mL) was kept stirring at rt for *z* h. Next, the solvent was removed under reduced pressure co-evaporating with toluene several times to give the unprotected thiohydantoins as α/β mixtures.

*4.3.1. (5S,6R,9R,10S)-6,7,10-Trihydroxy-9-hydroxymethyl-3-phenyl-2-thioxo-8-oxa-1,3-diazaspiro[4.5]decan-4-one (**30**).* *x*=47 mg (0.11 mmol); *y*=5 mL; *z*=5 h. Amorphous solid (35 mg, 92%). $[\alpha]_D^{23}$ +11 (*c* 1.01, CH_3OH). ^1H NMR (300 MHz, CD_3OD) β anomer: δ 7.45–7.36 (m, 3H, Ar), 7.25–7.22 (m, 2H, Ar), 5.30 (d, 1H, $J_{6,7}$=8.1 Hz, H-7), 4.17 (dd, 1H, $J_{9,10}$=10.2 Hz, $J_{9,1'a}$=0 Hz, $J_{9,1'b}$=4.8 Hz, H-9), 3.83 (d, 1H, $J_{1a',1'b}$=11.7 Hz, H-1′a), 3.75 (d, 1H, H-10), 3.64 (dd, 1H, H-1′b), 3.47 (d, 1H, H-6); α anomer: δ 5.09 (d, 1H, $J_{6,7}$=4.7, H-7), 3.88 (d, 1H, H-6); ^{13}C NMR (75.5 MHz, CD_3OD) β anomer: δ 185.8 (C-2), 174.5 (C-4), 135.0, 129.8, 129.7, 129.6 (Ar), 94.9 (C-7), 74.8, 74.7 (C-9, C-5), 74.4 (C-6), 70.3 (C-10), 62.6 (C-1′); α anomer: δ 94.6 (C-7), 70.1 (C-6); HRCI-MS *m*/*z* calcd for $C_{14}H_{17}N_2O_6S$, $[M+H]^+$: 341.0807, found: 341.0800.

*4.3.2. (5S,6R,9R,10S)-3-Butyl-6,7,10-trihydroxy-9-hydroxymethyl-2-thioxo-8-oxa-1,3-diazaspiro[4.5]decan-4-one (**31**).* *x*=141 mg (0.35 mmol); *y*=15 mL; *z*=6 h. Amorphous solid (142 mg, 88%). $[\alpha]_D^{23}$ +25 (*c* 0.92, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6): β anomer: 10.10 (s, 1H, NH), 7.30–5.70 (m, 4H, 4OH), 5.04 (d, 1H, $J_{6,7}$=7.8 Hz, H-7), 3.90 (ddd, 1H, $J_{9,10}$=9.9 Hz, $J_{9,1'a}$=2.0, $J_{9,1'b}$=5.4 Hz, H-9) 3.66–3.63 (m, 1H, H-1′a), 3.60–3.56 (m, 2H, H-1″), 3.47 (d, 1H, H-10), 3.37 (dd, 1H, $J_{1'a,1'b}$=12.0 Hz, H-1′b), 3.24 (d, 1H, H-6), 1.56–1.44 (m, 2H, H-2″), 1.35–1.19 (m, 2H, H-3″), 0.85 (t, 3H, $J_{H,H}$=7.3 Hz, CH_3); α anomer: δ 10.36 (s, 1H, NH), 4.90 (d, 1H, $J_{6,7}$=3.2 Hz, H-7), 3.91–3.85 (m, 1H, H-9), 0.86 (t, 3H, $J_{H,H}$=7.3 Hz, CH_3); ^{13}C NMR (75.5 MHz, DMSO-d_6): β anomer: δ 182.9 (C-2), 173.3 (C-4), 93.4 (C-7), 73.5 (C-9), 72.5 (C-6), 72.2 (C-5), 68.2 (C-10), 60.8 (C-1′), 40.3–38.6 (C-1″), 29.4, 19.1 ($2CH_2$), 13.7 (CH_3); α anomer: δ 182.5 (C-2), 176.4 (C-4), 92.8 (C-7), 74.1 (C-5), 68.0 (C-6), 67.9 (C-9), 67.3 (C-10), 60.3 (C-1′), 29.3 (CH_2); HRCI-MS *m*/*z* calcd for $C_{12}H_{21}N_2O_6S$, $[M+H]^+$: 321.1120, found: 321.1110.

*4.3.3. (5S,6R,9R,10S)-3-Cyclohexyl-6,7,10-trihydroxy-9-hydroxymethyl-2-thioxo-8-oxa-1,3-diazaspiro[4.5]decan-4-one (**32**).* *x*=140 mg (6.33 mmol); *y*=15 mL; *z*=3h. Amorphous solid (112.8 mg, 99%). $[\alpha]_D^{23}$ +22 (*c* 0.75, CH_3OH); ^1H NMR (500 MHz, DMSO-d_6): β anomer: δ 10.05 (s, 1H, NH), 6.74 (br s, 1H, OH-7), 5.64–5.60 (m, 2H, OH-6, OH-1′), 5.04 (d, 1H, $J_{6,7}$=8.0 Hz, H-7), 4.49 (br s, 1H, OH-10), 4.45–4.36 (m, 1H, H-1″), 3.90 (ddd, 1H, $J_{9,10}$=9.5 Hz, $J_{9,1'a}$=2.0 Hz, $J_{9,1'b}$=5 Hz, H-9), 3.61 (dd, 1H, $J_{1'a,1'b}$=12.0 Hz, H-1′a), 3.45 (dd, 1H, $J_{10,OH}$=5.0 Hz, H-10), 3.38 (dd, 1H- H-1′b), 3.22 (d, 1H, H-6), 2.14–2.09 (m, 2H, CH_2), 1.76–1.74 (m, 2H, CH_2), 1.61–1.47 (m, 4H, $2CH_2$), 1.25–1.17 (m, 2H, CH_2); α anomer: δ 10.33 (s, 1H, NH), 6.24 (d, 1H, $J_{7,OH}$=11.0 Hz, OH-7), 5.90 (d, 1H, $J_{10,OH}$=6.0 Hz, OH-10), 5.76 (br s, 1H, OH-6), 4.88 (dd, 1H, $J_{6,7}$=4.0 Hz, H-7), 4.45–4.36 (m, 1H, OH-1′), 3.86 (ddd, 1H, $J_{9,10}$=10.1 Hz, $J_{9,1'a}$=2.0 Hz, $J_{9,1'b}$=5.5 Hz, H-9), 3.65–3.62 (m, 1H, H-6), 3.59 (dd, 1H, $J_{1'a,1'b}$=12.0 Hz, H-1′a), 3.55 (dd, 1H, H-10), 3.48 (dd, 1H- H-1′b); ^{13}C NMR (125.7 MHz, DMSO-d_6): β anomer: δ 183.3

(C-2), 173.4 (C-4), 93.3 (C-7), 73.4 (C-9), 72.4 (C-6), 71.3 (C-5), 68.4 (C-10), 61.0 (C-1′), 53.6 (C-1″), 28.3, 25.5, 24.9 ($4CH_2$); α anomer: δ 182.9 (C-2), 176.7 (C-4), 92.8 (C-7), 73.1 (C-5), 68.0 (C-6), 67.7 (C-9), 67.5 (C-10), 60.4 (C-1′), 54.0 (C-1″), 28.1, 25.4, 24.8 ($3CH_2$); HRCI-MS *m*/*z* calcd for $C_{14}H_{23}N_2O_6S$, $[M+H]^+$: 347.1277, found: 347.1284.

*4.3.4. (5′S,6′R,9′R,10′S)-1,6-Bis-(6′,7′,10′-trihydroxy-9′-hydroxymethyl-4′-oxo-2′-thioxo-8′-oxa-1′,3′-diazaspiro[4.5]decan-3′-yl) hexane (**33**).* *x*=60 mg (0.08 mmol); *y*=6.4 mL; *z*=5 h. Amorphous solid (48 mg, 99%). $[\alpha]_D^{23}$ +15 (*c* 1.08, CH_3OH); ^1H NMR (500 MHz, CD_3OD) β anomer: δ 5.36 (d, 1H, $J_{6',7'}$=8.1 Hz, H-7′), 4.23 (ddd, 1H, $J_{9',10'}$=10.2 Hz, $J_{9',1''a}$=2.1 Hz, $J_{9',1''b}$=5.1 Hz, H-9′), 3.88 (dd, 1H, $J_{1''a,1''b}$=12.6 Hz, H-1″a), 3.83 (d, 1H, H-10′), 3.77 (t, 2H, $J_{1,2}$=6.9 Hz, H-1), 3.68 (dd, 1H, H-1″b), 3.56 (d, 1H, H-6′), 1.61 (quint, 2H, $J_{2,3}$=6.9 Hz, H-2), 1.33 (m, 2H, H-3); α anomer: δ 5.22 (d, 1H, $J_{6',7'}$=4.5 Hz, H-7′), 4.26 (m, 1H, H-9′) 4.02 (d, 1H, H-6′); ^{13}C NMR (75.5 MHz, D_2O) β anomer: δ 185.6 (C-2′), 174.9 (C-4′), 94.9 (C-7′), 73.7, 73.6 (C-9′, C-5′), 72.7 (C-6′), 68.7 (C-10′), 62.2 (C-1″), 41.4 (C-1), 27.3, 25.8 (C-2, C-3); α anomer: 93.1 (C-7′), 74.8 (C-5′), 68.0 (C-9′), 67.8 (C-10), 60.8 (C-1″); HRLSI-MS *m*/*z* calcd for $C_{22}H_{34}N_4O_{12}S_2Na$, $[M+Na]^+$: 633.1520, found: 633.1512.

*4.3.5. (5′S,6′R,9′R,10′S)-1,12-Bis-(6′,7′,10′-trihydroxy-9′-hydroxymethyl-4′-oxo-2′-thioxo-8′-oxa-1′,3′-diazaspiro[4.5]decan-3′-yl)dodecane (**34**).* *x*=108 mg (0.13 mmol); *y*=11.5 mL; *z*=5 h. Amorphous solid (86 mg, 98%). $[\alpha]_D^{23}$ +7 (*c* 1.30, CH_3OH); ^1H NMR (500 MHz, CD_3OD) β anomer: δ 5.30 (d, 1H, $J_{6',7'}$=8.0 Hz, H-7′), 4.17 (ddd, 1H, $J_{9',10'}$=10.0 Hz, $J_{9',1''a}$=2.3 Hz, $J_{9',1''b}$=5.0 Hz, H-9′), 3.82 (dd, 1H, $J_{1''a,1''b}$=12.0 Hz, H-1″a), 3.73 (t, 2H, $J_{1,2}$=7.2 Hz, H-1), 3.51 (d, 1H, H-10′), 3.62 (dd, 1H, H-1″b), 3.37 (d, 1H, H-6′), 1.64–1.61 (m, 2H, CH_2), 1.32–1.28 (m, 8H, $4CH_2$); α anomer: δ 5.05 (d, 1H, $J_{6',7'}$=4.5 Hz, H-7′), 4.13 (ddd, 1H, $J_{9',10'}$=10.0 Hz, $J_{9',1''a}$=3.0 Hz, $J_{9',1''b}$=4.5 Hz, H-9′); ^{13}C NMR (125.7 MHz, CD_3OD) β anomer: δ 186.2 (C-2′), 175.0 (C-4′), 94.9 (C-7′), 74.8 (C-9′), 74.3 (C-5′), 74.1 (C-6′), 70.1 (C-10′), 62.7 (C-1″), 41.7 (C-1), 30.7, 30.6, 30.4, 28.7, 27.6 (C-2–C-6); α anomer: δ 94.8 (C-7), 75.7 (C-5′), 69.3 (C-9), 64.9 (C-1″); HRLSI-MS *m*/*z* calcd for $C_{28}H_{46}N_4NaO_{12}S_2$, $[M+Na]^+$: 717.2451, found: 717.2464.

*4.3.6. (5′S,6′R,9′R,10′S)-Tris[2-(6′,7′,10′-trihydroxy-9′-hydroxymethyl-4′-oxo-2′-thioxo-8′-oxa-1′,3′-diazaspiro[4.5]decan-3′-yl) ethyl]ammonium trifluoroacetate(**35**).* *x*=102 mg (0.09 mmol); *y*=11.5 mL; *z*=5 h. Amorphous solid (78 mg, 98%). $[\alpha]_D^{23}$ +14 (*c* 1.00, CH_3OH); ^1H NMR (300 MHz, D_2O) β anomer: δ 5.34 (d, 1H, $J_{6',7'}$=8.1 Hz, H-7′), 4.29 (t, 2H, $J_{1,2}$=6 Hz, H-1), 4.21 (dd, 1H, $J_{9',10'}$=9.9 Hz, $J_{9'-1''a}$=0.0 Hz, $J_{9'-1''b}$=5.1 Hz, H-9′), 3.87 (d, 1H, $J_{1''a,1''b}$=12.6 Hz, H-1″a), 3.84 (d, 1H, H-10′), 3.68 (dd, 1H, H-1″b), 3.73–3.69 (m, 2H, H-2), 3.57 (d, 1H, H-6′); α anomer: δ 5.24 (d, 1H, $J_{6',7'}$=4.8 Hz, H-7′), 4.00 (d, 1H, H-6′); ^{13}C NMR (75.5 MHz, D_2O) β anomer: δ 183.2 (C-2′), 173.6 (C-4′), 163.5 (q, $^2J_{C,F}$=35.6 Hz, CF_3COO^-), 116.9 ($J_{C,F}$=291.6 Hz, CF_3COO^-), 92.9 (C-7′), 73.6, 73.0 (C-5′, C-9′), 72.1 (C-6′), 68.1 (C-10′), 60.5 (C-1″), 51.2 (C-2), 35.4 (C-1); HRLSI-MS *m*/*z* calcd for $C_{30}H_{45}N_7NaO_{18}S_3$ $[M-TFA+Na]^+$: 910.1881, found: 910.1838.

4.4. Enzymatic assays

Commercially-available glycosidases α-glucosidase (baker's yeast), β-glucosidase (almonds), β-galactosidase (*A. oryzae*) and β-galactosidase (*E. coli*) were used as received (Sigma–Aldrich) without further purification. The enzymatic assays were carried out as described previously.[33] Each glycosidase assay was performed by preparing ten 2-mL samples in PS cuvettes containing 0.1 M phosphate buffer (pH 6.8) and substrate solution (*p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside or *o*-nitrophenyl-β-D-galactopyranoside). The concentration of the substrate ranged from 0.25 to 4.0 K_m. Water or inhibitor solution plus water were also added up to a constant volume of 1.9 mL for the K_m or the K_i measurement, respectively. Reaction was started by adding 0.1 mL of dilute enzyme solution at 25 °C and the formation of the *p*- or *o*-nitrophenolate was monitored for 2 min by measuring the increase of absorbance at 400 or 420 nm, respectively. Initial rates were calculated from the slopes of each reaction and were used to obtain two Hanes plots ([*S*]/*V* vs [*S*]), one with and one without inhibitor. Inhibition constants (K_i) were obtained from the formula $K_i=[I]/(K_m'/K_m-1)$, were [*I*] is the inhibitor concentration in the cuvette and K_m and K_m' are the enzymatic Michaelis–Menten constants in the absence and in the presence of the inhibitor, respectively.

Glycogen phosphorylase activities were assayed in the direction of glycogen synthesis[34] by using commercial glycogen phosphorylase b (rabbit muscle, 10 μg/mL) at 30 °C in the presence of 1% oyster glycogen, α-D-glucose-1-phosphate, 1 mM AMP, with or without inhibitors at pH 6.8 (50 mM triethanolamine/HCl, 1 mM dithiothreitol and 1 mM EDTA buffer). P_i concentration was determined using the method developed by Taussky and Shorr.[35] Hanes plots were built using the initial velocity from each reaction, as indicated above for glycosidases.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.09.109.

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