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Synthesis of conformationally-constrained thio(seleno)hydantoins and α-triazolyl lactones from D-arabinose as potential glycosidase inhibitors

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ABSTRACT

We have explored the rich structural diversity provided by an α-azido ester derived from D-arabinose as the source of sugar templates with reduced conformational flexibility. Using transient α-thioureido(selenoureido) esters we have prepared spiranic thio(seleno)hydantoins at the C-3 position of the sugar moiety. In this context, the first example of a stable spiranic α-lactam (or aziridinone) was isolated as a by-product in the hydrogenolysis of the starting α-azido ester. Furthermore, using copper(I)-catalyzed azido–alkyne cycloaddition (*click chemistry*), we have accessed bicyclic *cis*-fused α-triazolyl lactones fixed in the furanose form. Spiranic thiohydantoins turned out to be moderate, though selective, inhibitors of glycosidases, whereas their selenium isosters behaved as good free radical scavengers.

1. Introduction

Incorporation of heterocyclic moieties into carbohydrate-based scaffolds is a widely used procedure in the preparation of biologically active compounds, such as immunostimulating agents,[1] antitumour[2] or antimicrobial derivatives.[3]

Among the plethora of carbohydrates containing heterocycles reported so far, a strategy considered for the improvement of the activities exhibited by such compounds consists in locking the conformation of the sugar residue as a way of attempting a better binding with the corresponding targeted biomolecules involved in their action.[4–8] In this context, it is worth mentioning spiro-annelated and bicyclic-fused carbohydrates. The former emerged after the isolation in 1991 of hydantocidin, the first known natural spiro-sugar,[9] exhibiting herbicidal and plant regulation activities with reduced toxicity towards mammals.[10] The design of analogous structures to hydantocidin has allowed the development of very potent inhibitors of glycogen phosphorylase,[11,12] a potential target in the treatment of type 2 *diabetes mellitus*.[13]

The number of synthetic carbohydrates containing spiroheterocyclic motifs, not only (thio)hydantoins, has increased incessantly since the isolation of hydantocidin, bearing the heterocyclic motifs mainly on positions C-1,[14] but also on C-2,[15] C-3[16] or C-4;[17] position C-3 is relatively less explored when compared with the anomeric carbon.

2. Results and discussion

In the search for new biologically active carbohydrate derivatives, we have explored the synthesis of conformationally-constrained architectures hold in locked conformations, and evaluated them as potential glycosidase inhibitors.

Herein we have exploited the structural diversity provided by D-*arabino*-configured α-azido ester **4**, which has been efficiently transformed into spiro-annelated thiohydantoins, selenohydantoins and an α-lactam, together with *cis*-fused bicyclic α-triazolyl lactones. Our main target is to compare the biological activity of these compounds with the previously reported by us starting from D-glucose;[18] on the one hand, the compounds presented in this manuscript have reversed stereochemistry on the spiranic centre, and on the other hand, the lack of a hydroxymethyl moiety might confer a different conformational behaviour of the sugar ring when compared with the glucose counterparts. Therefore these

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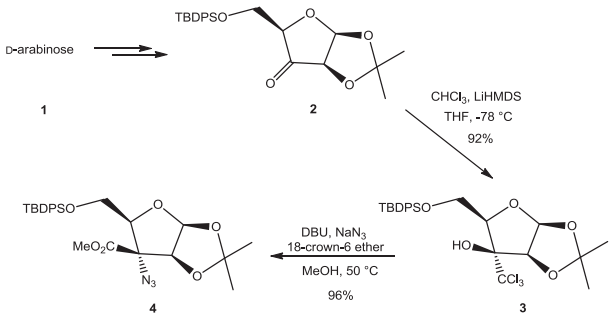
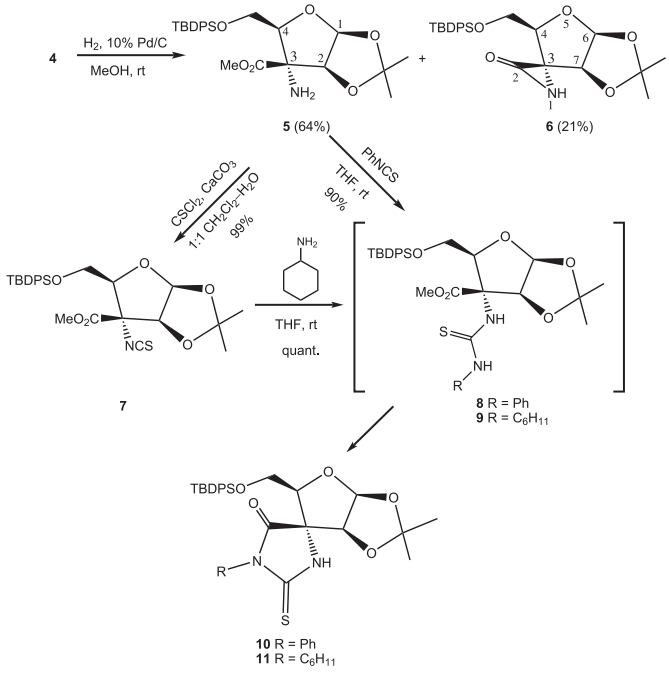
two aspects covered herein might modify the inhibitory properties of the targeted compounds.

Starting from commercially-available D-arabinose, the stereoselective synthesis of trichloromethyl derivative **3** has been reported from the corresponding ulose **2**;[19] subsequent modified Corey–Link reaction[20] of **3** with NaN_3, and DBU in MeOH furnished α-azido ester **4** (Scheme 1), as reported by Sørensen et al.[21] Compound **4** turned out to be a versatile starting material for the synthesis of an ample number of spiro-annelated and other bicyclic sugar derivatives, as demonstrated in this contribution.



Scheme 1.

Catalytic hydrogenation of α-azido ester **4** using Pd/C as catalyst yielded a separable mixture of two compounds (Scheme 2): α-aminoester **5** (64%) and unexpected spiranic α-lactam (or aziridinone) **6** (21%). NMR data indicated the lack of the methoxycarbonyl group in **6**, and a strong deshielding of C-3 (9.7 ppm) in comparison with α-aminoester **5**; these data are in agreement with a spirocyclization taking place.[16c]



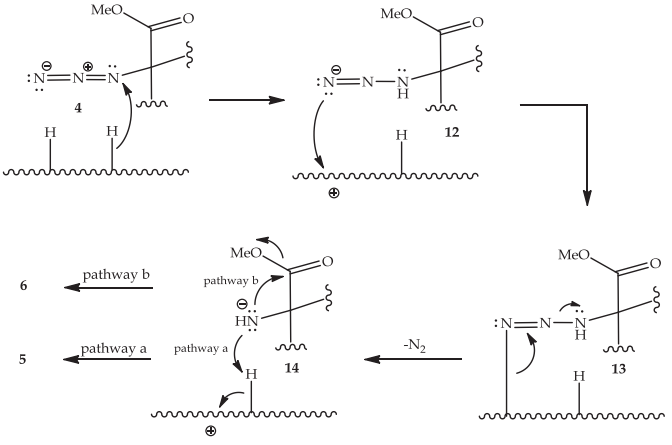
Scheme 2.

In the literature, reported data on α-lactams are scarce;[22] such compounds are usually obtained by treatment of *N*-substituted α-halo(mesyloxy)amides with strong bases like ^{t}BuOK or NaH under anhydrous conditions;[23,24] this family of strained compounds has received attention because of the regioselective ring-opening reactions exerted by certain nucleophiles,[23] leading to functionalized derivatives. Nevertheless, most of α-lactams are highly unstable compounds, and could only be isolated[25] when bearing a tertiary alkyl substituent on the nitrogen atom and at least one tertiary alkyl group on C-3.[26]

Surprisingly, aziridinone **6**, the first example of a spiranic α-lactam, turned out to be a highly stable compound, despite bearing no substituents on the nitrogen atom, and could even be isolated by column chromatography and characterized by NMR spectroscopy without any decomposition. Probably, the prominent steric hindrance exerted by the *tert*-butyldiphenylsilyloxy group, together with the dioxolane moiety precluded degradation of the aziridinone.

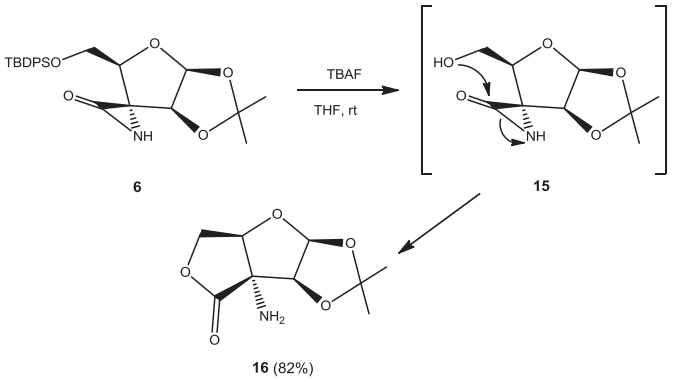
Attempts to convert aminoester **5** into aziridinone **6** using both acidic and basic catalysis at different temperatures and solvents were unsuccessful. Changes in solvent polarity, pH and temperature of the hydrogenolysis reaction led to no changes in the ratio of aminoester **5** and aziridinone **6**. Based on these results, we propose that α-lactam formation takes place via an anionic amide intermediate **14** generated in the hydrogenolysis reaction of azide **4**.

We postulate that intramolecular acyl nucleophilic substitution of transient **14** would lead to **6** (Scheme 3, pathway b), whereas capture of a proton (Scheme 3, pathway a) would lead to the expected aminoester **5**.



Scheme 3.

We attempted the deprotection of aziridinone **6** by treatment with a TBAF solution; removal of the TBDPS protective group led not to expected monohydroxylated derivative **15**, but to *cis*-fused α-amino-γ-lactone **16** in an 82% yield. The formation of such a compound can be explained considering a spontaneous nucleophilic attack of C-5 hydroxyl group on the carbonyl group, affording opening of the lactam moiety (Scheme 4). Lactones are valuable intermediates in organic synthesis, and in particular, bicyclic carbohydrate 1,2-lactones have been subjected to stereoselective opening with hetero- and *C*-nucleophiles to furnish functionalized carbohydrates.[27]



Scheme 4.

The structure of amino lactone **16** was further confirmed by X-ray diffraction[†] (Fig. 1).

Treatment of aminoester **5** with thiophosgene and $CaCO_3$ in a mixture of CH_2Cl_2–H_2O afforded isothiocyanato ester **7** (Scheme 2) in a quantitative yield. Aminoester **5** and isothiocyanato ester **7** were coupled with phenyl isothiocyanate and cyclohexylamine, respectively, to afford spiranic thiohydantoins **10** and **11**, respectively (Scheme 2) via the corresponding transient thioureas **8** and **9**, which underwent intramolecular acyl nucleophilic substitution involving the NH group. In the case of thiohydantoin **11**, cyclization took place spontaneously, and thiourea **9** was not detected by TLC. On the contrary, regarding *N*-phenyl thiohydantoin **10**, its formation required addition of Et_3N and heating at 60 °C.

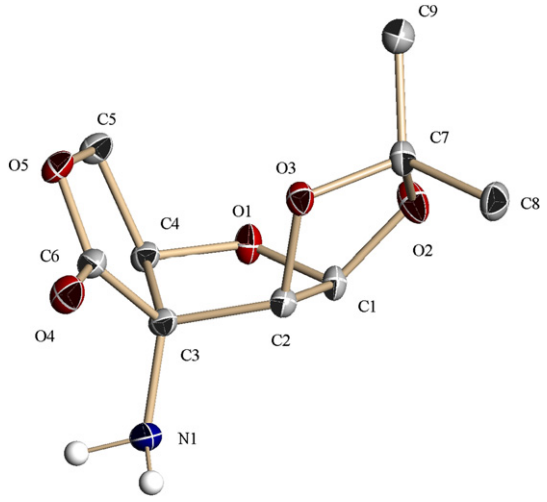
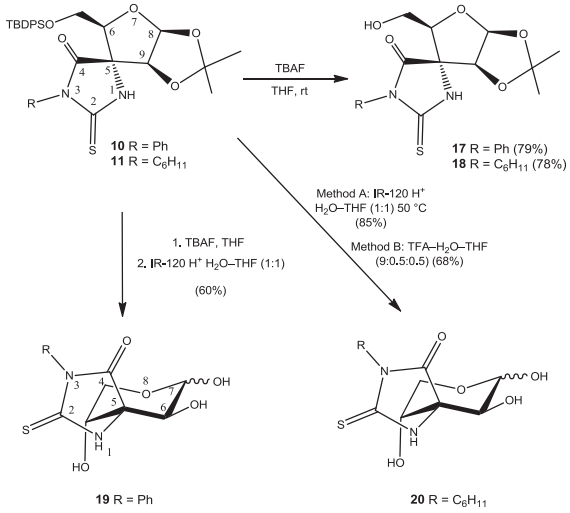


Fig. 1. ORTEP drawing of **16**.

NMR data of compounds **10** and **11** showed no signals for the methoxycarbonyl group; ^{1}H NMR spectra indicated a single signal for the NH group ranging from 8.27 to 8.89 ppm. Spiranic C-5 showed a deshielding (0.5–2.0 ppm) when compared with C-3 of aminoester **5**, demonstrating the presence of the spiranic ring.

We first attempted partial deprotection of compounds **10** and **11**, which was accomplished using a solution of TBAF, affording the elimination of the TBDPS group to give monohydroxylated thiohydantoins **17** and **18** (Scheme 5) in excellent yields (79 and 78%, respectively) after chromatographical purification. Fully deprotection of *N*-phenyl thiohydantoin **10** was achieved in two steps: firstly, treatment with TBAF, and subsequent addition of Amberlite IR-120(H^+) resin to the non-isolated monohydroxylated derivative **17** to give **19** with an overall yield of 60%.



Scheme 5.

Moreover, fully deprotection of *N*-cyclohexyl derivative **11** to furnish **20** was achieved using two procedures: treatment with acidic Amberlite IR-120(H^+) resin (85%) or, alternatively, with aqueous TFA (68%).

Both deprotected thiohydantoins **19** and **20** were obtained as anomeric mixtures of pyranosic and furanosic forms (Table 1). The major isomer of such compounds was assigned to the α pyranose form in a $^1C_4$ conformation, based on the $J_{6,7}$ coupling constant (6.6 and 6.3 Hz, respectively); on the other hand, for the second isomer in importance, the small value for the same coupling constant (roughly 3.3 Hz) confirmed the β anomer. Regarding the two minor isomers, the one with a higher frequency in the anomeric carbon (101.1 ppm for **19** and 100.8 ppm for **20**) was tentatively assigned[28] to the α furanose form.

Table 1
Anomeric ratio in $(CD_3)_2CO$ of compounds **19** and **20**

Compound	α-Pyranose	β-Pyranose	α-Furanose	β-Furanose
19	15.9	2.2	1	2.7
20	19.1	3.3	1	—[a]

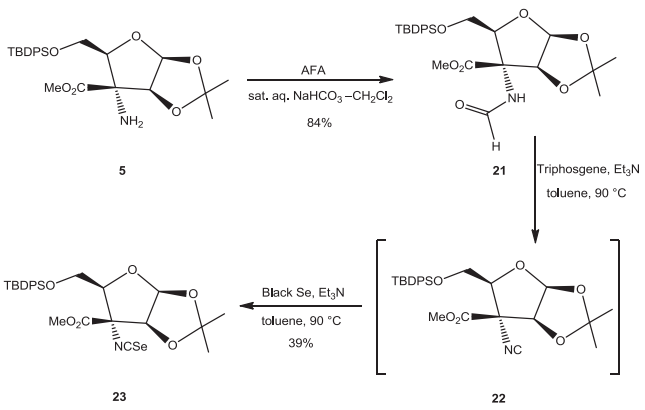
[a] Overlapping of signals.

The remarkable biological properties exhibited by organoselenium compounds, such as antioxidants,[29] antimicrobial,[30] anticancer[31] or anti-inflammatory agents,[32] among others, stimulated us to prepare easily-available seleno-isosters of the previous spiranic thiohydantoin templates in order to study their antioxidant properties.

For that purpose, we first attempted the preparation of the spiranic selenohydantoins by coupling α-isoselenocyanato ester **23** with different amines; such heterocumulene was accessed from aminoester **5** by formylation (Scheme 6) with freshly-prepared acetic-formic anhydride (AFA) in a biphasic satd aq

[†] Crystal data for **16**: $C_9H_{13}NO_5$, M=215.0, orthorhombic, a=6.2249(3) Å, b=7.0817(3) Å, c=21.6720(10) Å, α=90.00°, β=90.00°, γ=90.00°, V=955.36(8) Å^3, T=173(2) K, space group $P2(1)2(1)2(1)$, Z=4, μ(Mo Kα)=0.123 mm^{-1}, 10,227 reflections measured, 1662 independent reflections (R_{int}=0.0547). The final R_1 values were 0.0683 ($I>2\sigma(I)$). The final $wR(F^2)$ values were 0.1911 ($I>2\sigma(I)$). The final R_1 values were 0.0779 (all data). The final $wR(F^2)$ values were 0.2010 (all data). The goodness of fit on F^2 was 1.212. CCDC-859845 (**16**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

$NaHCO_3$–CH_2Cl_2 medium, following a procedure recently developed in our research group.[33] Formamide **21** was isolated in an 84% yield after chromatographical purification; ^1H NMR (8.13 ppm) and ^{13}C NMR spectra (160.2 ppm) confirmed the presence of the formamido moiety.



5 21 22 23

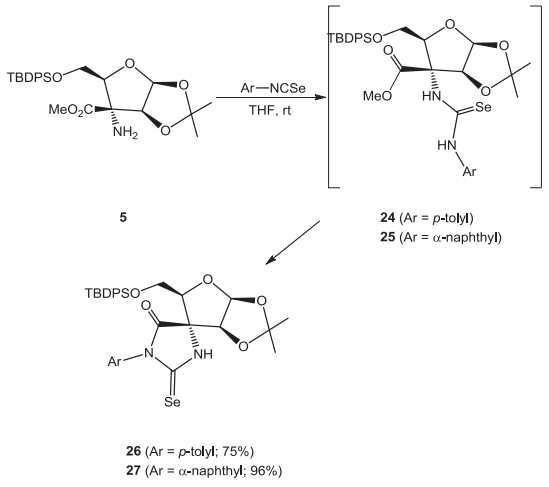
Scheme 6.

Compound **21** was dehydrated with triphosgene and Et_3N, under the same conditions used by our group in the preparation of sugar-derived isoselenocyanates,[33] to give transient non-isolated isocyanide **22**; such a compound was coupled with elemental black selenium at 90 °C in toluene to furnish α-isoselenocyanato ester **23** (Scheme 6) in a modest yield (39%, two steps), coming from partial decomposition under the reaction conditions.

The presence of the selenium atom was evidenced from the ^{13}C NMR spectrum, which showed resonance of the –N=C=Se group at 134.3 ppm, with a shielding of 6.2 ppm when compared with the thio-isoster **7**, in agreement with isoselenocyanates data.[33]

Although selenohydantoins can be obtained by direct coupling of **23** with different amines, considering the low yield in the preparation of the isoselenocyanate, even lower at a higher scale, we decided to modify the synthetic pathway in order to synthesize selenohydantoins in a more acceptable overall yield. In that sense, we decided to couple α-aminoester **5** with *p*-tolyl and α-naphthyl isoselenocyanates.[33]

Such aryl isoselenocyanates were coupled with **5** under mild conditions to furnish transient non-detected selenoureas **24** and **25**, which underwent a spirocyclization involving the methoxycarbonyl group leading to spiroselenohydantoins **26** and **27** (Scheme 7). The absence of signals in ^1H and ^{13}C NMR spectra regarding the methoxycarbonyl group confirms the cyclization reaction.



5 24 (Ar = *p*-tolyl) 25 (Ar = α-naphthyl)

26 (Ar = *p*-tolyl; 75%)
27 (Ar = α-naphthyl; 96%)

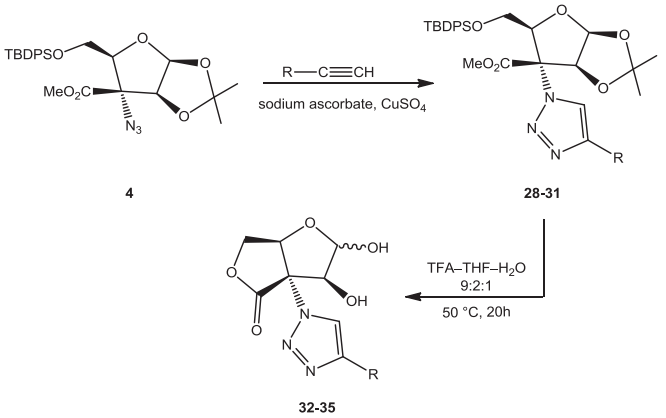
Scheme 7.

These are the first examples of both, sugar-derived selenohydantoins, and spiranic selenohydantoins.

Attempts to deprotect **26** or **27** failed, and extensive decomposition was observed, with release of elemental selenium.

We have also exploited the versatility offered by the α-azido ester **4** as a building block for the incorporation of triazolyl moieties via Cu(I)-catalyzed azide–alkyne cycloadditions, that is, *click chemistry* reactions.[34] Triazolyl moiety is a robust scaffold, compatible with a plethora of functional groups, and virtually chemically inert,[35] and has found numerous applications in Medicinal Chemistry, Materials Science, or liquid crystals, among others.

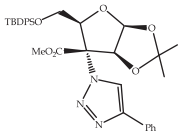
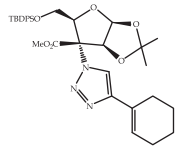
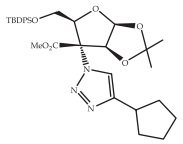
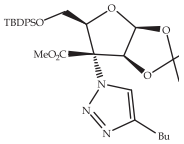
In this context, we have accomplished the preparation of 4-alkyl and aryl *arabino*-derived triazoles from **4** using commercially-available alkynes and sodium ascorbate/$CuSO_4$ as the Cu(I) source (Scheme 8); triazolyl esters **28**–**31** were obtained in a 62–85% yield after chromatographical purification (Table 2).



4 28-31 32-35

Scheme 8.

Table 2
Synthesis of α-triazolyl esters **28**–**31**

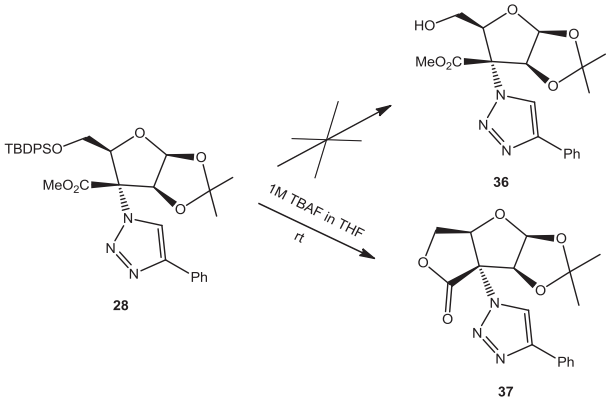
Entry	Alkyne	Triazole	Compd	Yield[a] (%)
1			**28**	62
2			**29**	66
3			**30**	85
4			**31**	76

[a] Isolated yield.

Carbohydrates bearing a triazolyl moiety on C-3 of D-*xylo*, D-*galacto*, D-*allo* and D-*gluco* configurations were reported to be α-glucosidase inhibitors, even bearing protective groups on the hydroxyl groups.[36] In our case, it was our intention to study how a bicyclic lactone could modulate the biological activity.

^{1}H NMR spectra of compounds **28**–**31** showed a singlet in the range 8.02–7.52 ppm, which is consistent with the proton H-5 of the triazolyl moiety, in agreement with reported data on triazole-containing carbohydrates.[36]

Triazole **28** was treated with a solution of TBAF in THF in order to obtain partially-deprotected derivative **36** (Scheme 9). Nevertheless, the ^{1}H NMR spectrum showed absence of the methoxycarbonyl moiety, and a remarkable difference between coupling constants $J_{4,5a}$ and $J_{4,5b}$; these data suggest instead the formation of *cis*–*cis*-arranged tricyclic lactone **37**, resulting from nucleophilic attack of C-5 hydroxyl group on the carbonyl group, maybe catalyzed by the TBAF present in the reaction medium.



Scheme 9.

The only two bibliographic reports concerning the synthesis of lactones on C-3 of a furanose involve either an intramolecular nucleophilic addition of an alkoxide on a cyano group, followed by hydrolysis of the transient imine,[37] or the intramolecular condensation between hydroxyl and amido groups.[38]

Unfortunately, attempts to purify derivative **37** from TBAF by column chromatography were unsuccessful.

Alternatively, we tried to eliminate tetrabutylammonium salts following a recent reported procedure, based on treatment with $CaCO_3$ and Dowex 50W resin;[39] nevertheless, although the ratio of the tetrabutylammonium was reduced, it could not be eliminated completely. Subsequently, in order to enable the purification process, we decided to remove simultaneously both, the TPDPS and isopropylidene groups, using an aqueous TFA solution.

Treatment of triazole **28** with a 9:1:1 TFA–H_2O–THF mixture at rt afforded a complex mixture, as indicated by NMR spectrum (Fig. 2A); the presence of a series of singlets in the region 3.60–3.75 ppm, presumably corresponding to methoxycarbonyl groups suggests the existence of anomeric mixtures of non-lactonized pyranosic and furanosic forms **38** and **39**, together with bicyclic lactone **32** (Scheme 10).

In the deprotection of **28**, a gradual increase of temperature led to a simplification of the ^{1}H NMR spectrum in the sugar region (5.30–4.60 ppm), and to the disappearance of the signals around 3.60–3.75 ppm of the methoxycarbonyl group (Fig. 2B–D). In an additional assay, triazole **28** was heated at 50 °C in acidic medium for 20 h; subsequent purification by gel permeation chromatography (Biogel P2) afforded exclusively unprotected triazolyl lactone **32** as a 1:1 anomeric mixture (Scheme 8).

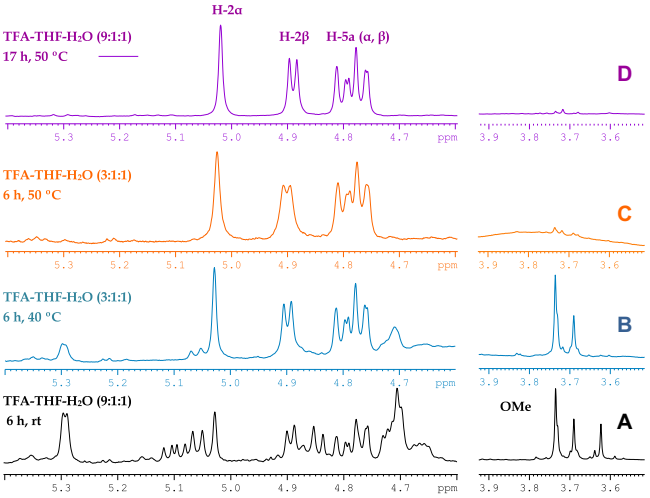
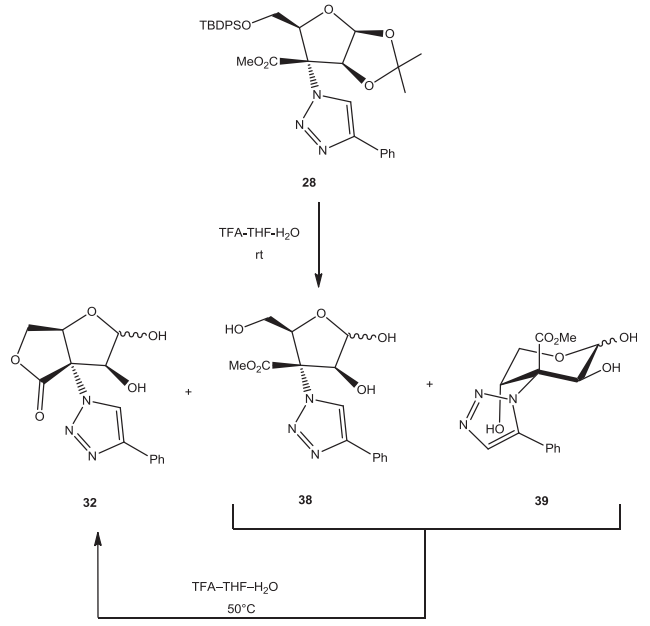


Fig. 2. Selected ^{1}H NMR regions for the deprotection of **28**.



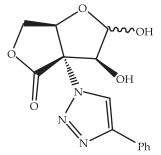
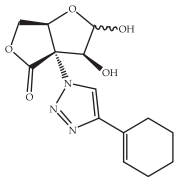
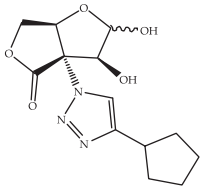
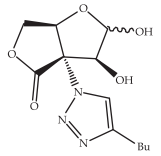
Scheme 10.

This successful procedure, yielding exclusively *cis*-fused bicyclic lactones, was also applied to triazoles **29**–**31**, under the same reaction conditions, to afford unprotected α-triazolyl lactones **33**–**35**, from moderate to excellent yields (Table 3), as anomeric mixtures (Scheme 8) in roughly 1:1 ratio (Table 4).

Coupling constant $J_{1,2}$ for the α anomer was found to be roughly 0 Hz in all the unprotected triazolyl lactones, whereas for the β isomer it ranged from 3.3 to 4.2 Hz. The unequivocal assignment of the complete spin system for both anomers was accomplished using HMBC experiments. The strong differences found for coupling constants $J_{4,5a}$, $J_{4,5b}$ in **32**–**35** ($J_{4,5a}$ ~ 6 Hz, $J_{4,5b}$ 0–2.7 Hz) when compared with non-lactonized derivatives **28**–**31** ($J_{4,5a}$=$J_{4,5b}$=6.3–6.2 Hz) are compatible with the lack of conformational freedom characteristic of a bicyclic system.

Regarding ^{13}C NMR spectra, carbonyl groups of lactones **32**–**35** (181.2–169.8 ppm) showed deshielding when compared with their ester counterparts **28**–**31** (166.0–165.1 ppm); similarly, C-5 of the unprotected triazolyl lactones also showed a deshielding of roughly 10 ppm compared with protected compounds.

Table 3
Deprotection of compounds **28**–**31**

Compound	Triazole	Yield[a] (%)
28		86
29		50
30		64
31		36

[a] Isolated yield.

3. Biological activities

3.1. Glycosidase inhibition

Thiohydantoins **19**, **20** and α-triazolyl lactones **32**–**35** were tested as potential glycosidase inhibitors against five commercially-available glycosidases (α-glucosidase from baker's yeast, β-glucosidase from almonds, α-galactosidase from green coffee beans, β-galactosidase from *Aspergillus oryzae* and β-galactosidase from *Escherichia coli*), and against glycogen phosphorylase type b from rabbit muscle.

Cyclohexyl thiohydantoin **20** was found to be a moderate, though selective, inhibitor of α-galactosidase (K_i=160 μM), and phenyl derivative **19** exhibited weak inhibition against glycogen phosphorylase (K_i=910 μM). Regarding triazole derivatives, only cyclohexenyl derivative **33** exhibited very weak inhibition against α-glucosidase (10% inhibition at 404 μM). These results, although moderate, are much better than those obtained for glucose counterparts.[18]

3.2. Antioxidant activity

We tested the radical-scavenging activity of spiranic selenohydantoin **27**. In this context, DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable commercially-available free radical, which constitutes the basis for one of the most popular assays for testing the radical-scavenging activity.[40] The methanolic solution of this compound, of a deep purple colour, exhibits a strong absorption at 515 nm, which is reduced in the presence of an antioxidant agent to give a yellowish solution. The remaining concentration of DPPH is, therefore, inversely proportional to the free radical-scavenging activity exhibited by the antioxidant agent.[41]

The calculated EC_{50} value for spiranic selenohydantoin **27**, that is, the concentration of the antioxidant agent required for reducing 50% of the initial DPPH concentration, was found to be 87±15 μM; this value is quite similar to that reported[40] for BHT (butylhydroxytoluene or 2,6-di-*tert*-butyl-4-methylphenol), a synthetic industrial antioxidant agent (EC_{50}=60 μM).

Table 4
Anomeric ratio in compounds **32**–**35**

Compd	**32**	**33**	**34**	**35**
α/β ratio	1:1[a]	1:1[a]	1:1[a] 3:1[b]	1:1[a]

[a] In $(CD_3)_2CO$.
[b] In DMSO-d_6.

4. Conclusions

In conclusion, we have demonstrated the high chemical versatility of a D-*arabino*-configured α-azido ester template by efficiently transforming it into novel conformationally-locked structures with potential biological activity: spiranic thio- and selenohydantoins, via spirocyclization involving an α-thioureido or selenoureido ester, or bicyclic *cis*-fused α-triazolyl lactones, through *click chemistry reactions*. An hitherto unknown and stable spiranic α-lactam was also isolated as a by-product. One of the thiohydantoins behaved as a moderate inhibitor of α-galactosidase, whereas protected selenohydantoins behaved as good free radical scavengers.

5. Experimental section

5.1. 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, with 3% ninhydrin in EtOH or with vanillin (1.5% in EtOH in the presence of 1% H_2SO_4). Column chromatography was performed using E. Merck Silica Gel 60 (40–63 μm).

*5.1.1. 3-Amino-3-deoxy-5-O-tert-butyldiphenylsilyl-1,2-O-isopropylidene-3-C-methoxycarbonyl-β-D-arabinofuranose (**5**) and (3R,4S,6S,7S)-4-tert-butyldiphenylsilyloxymethyl-6,7-(dimethylmethylenedioxy)-5-oxa-1-azaspiro[2.4]heptan-2-one (**6**).* To a solution of azidoester **4** (300 mg, 0.59 mmol) in methanol (23 mL) was added 10% Pd/C (90 mg) and the mixture was hydrogenated at atmospheric pressure and rt for 30 min. Then, the reaction was filtered over a Celite pad and the filtrate was concentrated to dryness to give a mixture of compounds **5** and **6** that were separated by column chromatography (9:1→3:2 hexane–EtOAc).

Eluted first was **6**: 56 mg, 21%; R_f 0.74 (3:2 hexane–EtOAc); $[\alpha]_D^{20}$ +10 (*c* 1.16, CH_2Cl_2); IR ν_{max} 3461, 3009, 2970, 1739, 1439, 1365, 1228, 1208 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.58 (s, 1H, NH), 7.63–7.57 (m, 4H, Ar–H), 7.42–7.35 (m, 6H, Ar–H), 6.06 (d, 1H, $J_{6,7}$=4.2 Hz, H-6), 4.82 (d, 1H, H-7), 4.56 (dd, 1H, $J_{4,1'a}$=9.4 Hz, $J_{4,1'b}$=4.3 Hz, H-4), 4.20 (t, 1H, $J_{1'a,1'b}$=9.3 Hz, H-1′a), 3.84 (dd, 1H, H-1′b), 1.59, 1.36 (2s, 3H each, $2CH_3$), 1.00 (s, 9H, $C(CH_3)_3$); ^{13}C NMR

(75.5 MHz, $CDCl_3$): δ 172.0 (C-2), 135.6, 132.8, 132.7, 130.1, 130.0, 128.0, 127.0 (Ar–C), 116.5 (*C*Me_2), 106.2 (C-6), 85.5 (C-7), 81.5 (C-4), 77.4 (C-3), 61.3 (C-1′), 27.4, 27.1 ($2CH_3$), 26.7 (C(*C*H_3)$_3$), 19.2 (*C*$(CH_3)_3$); Anal. Calcd for $C_{25}H_{31}NO_5Si$: C, 66.20; H, 6.89; N, 3.09, found: C, 66.27; H, 6.95; N, 3.30.

Eluted second was **5**: 182 mg, 64%; R_f 0.34 (3:2 hexane–EtOAc); $[\alpha]_D^{24}$ −13 (*c* 1.02, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.65–7.60 (m, 4H, Ar–H), 7.42–7.34 (m, 6H, Ar–H), 5.84 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 4.49 (d, 1H, H-2), 4.16 (dd, 1H, $J_{4,5a}$=7.8 Hz, $J_{5a,5b}$=9.1 Hz, H-5a), 4.07 (dd, 1H, $J_{4,5b}$=5.7 Hz, H-4) 3.96 (dd, 1H, H-5b), 3.70 (s, 3H, OMe), 3.40 (br s, 2H, NH_2), 1.43, 1.30 (2s, 3H each, $2CH_3$), 1.03 (s, 9H, C(*C*H_3)$_3$)); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 171.4 (CO), 135.6 (×4), 133.0 (×2), 129.9 (×2), 127.9 (×4) (Ar–C), 115.6 (*C*Me_2), 104.9 (C-1), 90.1 (C-2), 85.7 (C-4), 67.7 (C-3), 63.2 (C-5), 52.1 (OMe), 27.1 (CH_3), 26.9 (×4) (CH_3, C(*C*H_3)$_3$), 19.2 (*C*$(CH_3)_3$); CIMS *m*/*z* 486 ([M+H]$^+$, 13%); HRCI-MS calcd for $C_{26}H_{36}NO_6Si$ ([M+H]$^+$): 486.2312, found: 486.2298.

*5.1.2. 5-O-tert-Butyldiphenylsilyl-3-deoxy-3-isothiocyanato-1,2-O-isopropylidene-3-C-methoxycarbonyl-β-D-arabinofuranose (**7**).* To a solution of aminoester **5** (37 mg, 0.08 mmol) in a 1:1 mixture of CH_2Cl_2–H_2O (4 mL), were added $CaCO_3$ (31.5 mg, 0.31 mmol) and thiophosgene (7.8 μL, 0.10 mmol), and the mixture was vigorously stirred for 20 min. Then, the salts were filtered off and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (2×25 mL), and the combined organic fractions were dried over $MgSO_4$, filtered and the filtrate was concentrated to dryness. The residue was purified by column chromatography (19:1 hexane–EtOAc) to give isothiocyanate **7** as a syrup: 40 mg, 99%; R_f 0.58 (4:1 hexane–EtOAc); $[\alpha]_D^{24}$ −36 (*c* 1.25, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68–7.64 (m, 4H, Ar–H), 7.44–7.36 (m, 6H, Ar–H), 5.85 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 4.79 (d, 1H, H-2), 4.24 (dd, 1H, $J_{4,5a}$=7.2 Hz, $J_{4,5b}$=6.0 Hz, H-4), 4.15 (dd, 1H, $J_{5a,5b}$=10.2 Hz, H-5a) 3.95 (dd, 1H, H-5b), 3.78 (s, 3H, OMe), 1.43, 1.33 (2s, 3H each, $2CH_3$), 1.06 (s, 9H, C(*C*H_3)$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 164.5 (C=O), 140.5 (NCS), 135.7 (×2), 133.2, 133.0, 129.9, 127.9 (×2) (Ar–C), 116.4 (*C*Me_2), 104.8 (C-1), 88.6 (C-2), 86.5 (C-4), 72.5 (C-3), 62.6 (C-5), 53.1 (OMe), 27.2 (CH_3), 26.8 (C(*C*H_3)$_3$), 26.8 (CH_3), 19.3 (*C*$(CH_3)_3$); LSIMS *m*/*z* 550 ([M+Na]$^+$, 78%); HRLSI-MS calcd for $C_{27}H_{33}NNaO_6SSi$ ([M+Na]$^+$): 550.1696, found: 550.1701.

*5.1.3. (5R,6S,8S,9S)-6-tert-Butyldiphenylsilyloxymethyl-8,9-(dimethylmethylenedioxy)-3-phenyl-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**10**).* To a solution of aminoester **5** (112 mg, 0.23 mmol) in THF (1.5 mL) was added phenyl isothiocyanate (41 μL, 0.34 mmol) and the mixture was stirred at rt for 16 h. After disappearance of the starting material, triethylamine (16 μL, 0.11 mmol) was added, and the solution was heated at 60 °C for 1 h. Then, the solvent was removed under reduced pressure and the residue was purified by column chromatography (4:1 hexane–EtOAc) to give thiohydantoin **10** as a foam: 123 mg, 91%; R_f 0.65 (3:2 hexane–EtOAc); $[\alpha]_D^{23}$ −36 (*c* 0.57, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.89 (s, 1H, NH), 7.66–7.61 (m, 4H, Ar–H), 7.45–7.28 (m, 11H, Ar–H), 5.88 (d, 1H, $J_{8,9}$=3.6 Hz, H-8), 4.84 (d, 1H, H-9), 4.33 (m, 2H, H-6, H-1′a), 3.87 (dd, 1H, $J_{6,1'b}$=3.3 Hz, $J_{1'a,1'b}$=9.0 Hz, H-1′b), 1.47, 1.31 (2s, 3H each, $2CH_3$), 1.00 (s, 9H, C(*C*H_3)$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 182.6 (C-2), 166.9 (C-4), 135.7, 133.0, 132.9, 132.6, 130.0 (×2), 129.4, 129.1, 128.6, 128.0, 127.9 (Ar–C), 116.1 (*C*Me_2), 105.1 (C-8), 87.7 (C-9), 84.9 (C-6), 69.9 (C-5), 62.0 (C-1′), 27.0, 26.7 ($2CH_3$, C(*C*H_3)$_3$), 19.3 (*C*(CH_3)); LSIMS *m*/*z* 611 ([M+Na]$^+$, 17%); HRLSI-MS calcd for $C_{32}H_{36}N_2NaO_5SSi$ ([M+Na]$^+$): 611.2012, found: 611.2026.

*5.1.4. (5R,6S,8S,9S)-6-tert-Butyldiphenylsilyloxymethyl-3-cyclohexyl-8,9-(dimethylmethylenedioxy)-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**11**).* To a solution of isothiocyanato ester **7** (75 mg, 0.14 mmol) in THF (1.5 mL) was added cyclohexylamine (24 μL, 0.21 mmol) and the mixture was stirred at rt for 1.5 h. After that, the solvent was removed under reduced pressure and the residue was purified by column chromatography (9:1 hexane–EtOAc) to give thiohydantoin **11** as a foam: 84 mg, quant.; R_f 0.31 (4:1 hexane–EtOAc); $[\alpha]_D^{23}$ −20 (*c* 0.99, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.27 (s, 1H, NH), 7.67–7.62 (m, 4H, Ar–H), 7.41–7.37 (m, 6H, Ar–H), 5.86 (d, 1H, $J_{8,9}$=4.0 Hz, H-8), 4.65 (d, 1H, H-9), 4.45 (tt, 1H, $J_{H,H}$=4.0 Hz, $J_{H,H}$=12.5 Hz, H-1″), 4.28 (dd, 1H, $J_{6,1'a}$=7.5 Hz, $J_{1'a,1'b}$=10.5 Hz, H-1′a), 4.19 (dd, 1H, $J_{6,1'b}$=5.0 Hz, H-6), 3.84 (dd, 1H, H-1′b), 2.30 (dq, 1H, $J_{H,H}$=12.8 Hz, $J_{H,H}$=4.0 Hz, $CH_{cyclohex.}$), 2.22 (dq, 1H, $J_{H,H}$=13.0 Hz, $J_{H,H}$=4.0 Hz, $CH_{cyclohex.}$), 1.85–1.59 (m, 6H, $CH_{cyclohex.}$), 1.46, 1.28 (2s, 3H each, $2CH_3$), 1.24–1.18 (m, 2H, $2CH_{cyclohex.}$), 1.03 (C(*C*H_3)$_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 183.4 (C-2), 167.6 (C-4), 135.7 (×2), 133.2, 133.1, 129.9 (×2), 127.9 (×2) (Ar–C), 115.7 (*C*Me_2), 105.1 (C-8), 87.2 (C-9), 85.5 (C-6), 68.2 (C-5), 62.3 (C-1′), 55.9 (C-1″), 28.9, 28.7 ($2CH_2$), 27.0 (*C*(CH_3)), 26.8, 26.5 ($2CH_3$), 26.0 (×2), 25.2 ($2CH_2$), 19.3 (*C*$(CH_3)_3$); LSIMS *m*/*z* 617 ([M+Na]$^+$, 44%); HRLSI calcd for $C_{32}H_{42}N_2NaO_5SSi$ ([M+Na]$^+$): 617.2481, found: 617.2488.

*5.1.5. 3-Amino-3-C-3,5-carbolactono-3-deoxy-1,2-O-isopropylidene-β-D-arabinofuranose (**16**).* To a solution of aziridinone **6** (230 mg, 0.51 mmol) in THF (2 mL) was added a 1 M solution of TBAF in THF (560 μL, 0.56 mmol), and the mixture was stirred at rt for 2 days. Then, the solvent was removed under reduced pressure, and the residue was purified by column chromatography (4:1 hexane–EtOAc→EtOAc) to give lactone **16** as an amorphous solid, which was recrystallized from ethanol: 91 mg, 82%; mp: 196–197 °C; R_f 0.24 (7:3 hexane–EtOAc); $[\alpha]_D^{26}$ −99 (*c* 0.56, CH_2Cl_2); IR ν_{max} 3440, 3014, 2969, 1738, 1370, 1222, 1139, 1105, 943 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.06 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 4.64 (dd, 1H, $J_{4,5a}$=7.9 Hz, $J_{4,5b}$=5.7 Hz, H-4), 4.46 (d, 1H, H-2), 4.42 (dd, 1H, $J_{5a,5b}$=9.7 Hz, H-5a), 4.06 (dd, 1H, H-5b), 2.47 (s, 2H, NH_2), 1.44, 1.24 (2s, 3H each, $2CH_3$); ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 175.2 (CO), 112.5 (*C*Me_2), 108.4 (C-1), 87.3 (C-2), 86.3 (C-4), 69.7 (C-5), 68.7 (C-3), 26.4, 25.6 ($2CH_3$); CIMS *m*/*z* 216 ([M+H]$^+$, 12%); HRCI-MS calcd for $C_9H_{14}NO_5$ ([M+H]$^+$): 216.0872, found: 216.0872; Anal. Calcd for $C_9H_{13}NO_5$: C, 50.23; H, 6.09; N, 6.51, found: C, 50.22; H, 6.08; N, 6.19.

5.2. General procedure for the partial deprotection of spirothiohydantoins 10 and 11

To a solution of thiohydantoin **10** or **11** (0.16 mmol) in THF (2.0 mL) was added a 1 M solution of TBAF in THF (190 μL, 0.19 mmol) and the mixture was stirred at rt during the time indicated in each case. Then, the solvent was removed under reduced pressure and the residue was purified by column chromatography (4:1→3:2 hexane–EtOAc) to give compounds **17** and **18** as foams.

*5.2.1. (5R,6S,8S,9S)-8,9-(Dimethylmethylenedioxy)-6-hydroxymethyl-3-phenyl-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**17**).* Stirring was kept for 24 h: 46 mg, 81%; R_f 0.10 (7:3 hexane–EtOAc); $[\alpha]_D^{23}$ −76 (*c* 1.30, CH_2Cl_2); ^{1}H NMR (300 MHz, CD_3OD): δ 7.53–7.41 (m, 3H, Ar–H), 7.32–7.28 (m, 2H, Ar–H), 5.96 (d, 1H, $J_{8,9}$=3.9 Hz, H-8), 4.85 (d, 1H, H-9), 4.21 (t, 1H, $J_{6,1'a}$=$J_{6,1'b}$=7.0 Hz, H-6), 3.93 (m, 2H, H-1′a, H-1′b), 1.58, 1.25 (2s, 3H each, $2CH_3$); ^{13}C NMR (75.5 MHz, CD_3OD): δ 183.8 (C-2), 170.0 (C-4), 134.9, 130.2, 130.1, 129.9 (Ar–C), 119.9 (*C*Me_2), 106.6 (C-8), 88.6 (C-9), 85.6 (C-6), 77.1 (C-5), 61.3 (C-1′), 28.0 (×2) ($2CH_3$); LSIMS *m*/*z* 373 ([M+Na]$^+$, 48%); HRLSI-MS calcd for $C_{16}H_{18}N_2NaO_5S$ ([M+Na]$^+$): 373.0834, found: 373.0832.

*5.2.2. (5R,6S,8S,9S)-3-Cyclohexyl-8,9-(dimethylmethylenedioxy)-6-hydroxymethyl-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**18**).* Stirring was kept for 48 h: 46 mg, 81%; R_f 0.11 (7:3 hexane–EtOAc); $[\alpha]_D^{23}$ −70 (*c* 1.25, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$):

δ 5.97 (d, 1H, $J_{8,9}$=3.6 Hz, H-8), 4.67 (d, 1H, H-9), 4.46 (tt, 1H, $J_{H,H}$=3.7 Hz, $J_{H,H}$=12.3 Hz, H-1″), 4.27 (t, 1H, $J_{6,1'a}$=$J_{6,1'b}$=6.5 Hz, H-6), 4.12 (m, 1H, H-1′a), 3.91 (dd, 1H, $J_{1'a,1'b}$=11.7 Hz, H-1′b), 2.34–2.03 (m, 2H, CH_2), 1.86–1.82 (m, 2H, CH_2), 1.68–1.65 (m, 3H, CH_2, $CH_{cyclohex.}$), 1.65, 1.32 (2s, 3H each, $2CH_3$), 1.40–1.21 (m, 3H, CH_2, $CH_{cyclohex.}$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 183.2 (C-2), 168.9 (C-4), 115.3 (CMe_2), 105.6 (C-8), 86.4 (C-9), 86.0 (C-6), 68.4 (C-5), 61.5 (C-1′), 56.1 (C-1″), 28.6 (CH_2), 28.5 (CH_3), 26.4 (CH_2), 26.3 (CH_2), 26.0 (×2) (CH_2, CH_3), 25.2 (CH_2); LSIMS *m*/*z* 379 ([M+Na]$^+$, 100%); HRLSI-MS calcd for $C_{16}H_{24}N_2NaO_5S$ ([M+Na]$^+$): 379.1304, found: 379.1309.

*5.2.3. (5R,6S,7R and 7S,10S)-6,7,10-Trihydroxy-3-phenyl-2-thioxo-8-oxa-1,3-diazaspiro[4.5]decan-4-one (**19p**) and (5R,6S,8R and 8S,9S)-8,9-dihydroxy-6-hydroxymethyl-3-phenyl-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**19f**).* To a solution of thiohydantoin **10** (121 mg, 0.20 mmol) in THF (2 mL) was added a 1 M solution of TBAF in THF (250 μL, 0.25 mmol) and the mixture was stirred at rt for 24 h. Then, H_2O (2 mL) and Amberlite IR-120(H^+) resin (164 mg) were added and the mixture was stirred at rt for 36 h. After that, the resin was filtered off, and the filtrate was concentrated to dryness; the residue was purified by gel permeation chromatography (Biogel P2) to give thiohydantoin **19** as an α/β mixture of pyranose and furanose forms: 38 mg, 60%; R_f 0.45 (EtOAc); $[\alpha]_D^{25}$ −38 (*c* 1.09, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 7.51–7.38 (m, 3H, Ar–H), 7.33–7.25 (m, 2H, Ar–H); α pyranose: δ 5.19 (d, 1H, $J_{6,7}$=6.6 Hz, H-7), 4.18 (dd, 1H, $J_{9a,10}$=1.8 Hz, $J_{9a,9b}$=12.3 Hz, H-9a), 4.12 (dd, 1H, $J_{9b,10}$=3.3 Hz, H-10), 3.99 (d, 1H, H-6), 3.89 (dd, 1H, H-9b); β pyranose: δ 5.13 (d, 1H, $J_{6,7}$=3.3 Hz, H-7), 4.29 (m, 1H, H-6), 4.23–4.20 (m, 1H, $J_{9a,10}$=2.4 Hz, H-9a), 3.72 (dd, 1H, $J_{9b,10}$=4.2 Hz, $J_{9a,9b}$=12.9 Hz, H-9b); α furanose: δ 5.55 (d, 1H, $J_{8,9}$=5.4 Hz, H-8), 4.44 (d, 1H, H-9), β furanose: δ 5.24 (d, 1H, $J_{8,9}$=3.3 Hz, H-8), 4.09–4.04 (m, 1H, H-1′a), 3.93 (d, 1H, H-9), 3.66–3.62 (m, 1H, H-1′b); ^{13}C NMR (75.5 MHz, CD_3OD): α pyranose: δ 184.2 (C-2), 173.7 (C-4), 134.8, 130.4, 129.5, 129.4 (Ar–C), 95.3 (C-7), 72.3 (C-6), 71.8 (C-5), 69.0 (C-10), 65.1 (C-9); β pyranose: δ 94.4 (C-7), 68.4 (C-10), 68.2 (C-6), 61.7 (C-9); α furanose: δ 101.1; β furanose: δ 92.6 (C-8), 69.8 (C-9), 59.3 (C-1′); LSIMS *m*/*z* 311 ([M+Na]$^+$, 16%); HRLSI-MS calcd for $C_{13}H_{15}N_2O_5S$ ([M+H]$^+$): 311.0702, found: 311.0690.

*5.2.4. (5R,6S,7R and 7S,10S)-3-Cyclohexyl-6,7,10-trihydroxy-2-thioxo-8-oxa-1,3-diazaspiro[4.5]decan-4-one (**20p**) and (5R,6S,8R and 8S,9S)-3-cyclohexyl-8,9-dihydroxy-6-hydroxymethyl-2-thioxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**20f**). Method A*: To a solution of thiohydantoin **18** (47 mg, 0.13 mmol) in a 1:1 THF–H_2O mixture (2 mL) was added Amberlite IR-120(H^+) resin (104 mg). The mixture was stirred at 50 °C for 24 h. Then, the resin was filtered off and the filtrate was concentrated to dryness to give thiohydantoin **20** as an amorphous solid, and as an α/β mixture of pyranose and furanose forms: 34 mg (85%).

Method B: To a solution of thiohydantoin **18** (45 mg, 0.12 mmol) in a 1:1 THF–H_2O mixture (1 mL) was added TFA (4.5 mL), and the mixture was stirred at rt for 6 h. Then, the solvent was removed under reduced pressure and co-evaporated with toluene several times. The residue was purified by gel permeation chromatography (Biogel P2) to give thiohydantoin **20** as an amorphous solid, and as an α/β mixture of pyranose and furanose forms: 26 mg, 68%; R_f 0.50 (EtOAc); $[\alpha]_D^{24}$ −42 (*c* 1.16, $(CH_3)_2CO$); ^{1}H NMR (300 MHz, $(CD_3)_2CO$) α pyran: δ 8.87 (br s, 1H, NH), 5.14 (d, 1H, $J_{6,7}$=6.3 Hz, H-7), 4.66 (m, 1H, OH), 5.40 (tt, 1H, $J_{H,H}$=3.6 Hz, $J_{H,H}$=12.6 Hz, H-1′), 4.12 (dd, 1H, $J_{9a,10}$=1.8 Hz, $J_{9a,9b}$=12.3 Hz, H-9a), 3.88 (dd, 1H, $J_{9b,10}$=3.0 Hz, H-10), 3.82 (d, 1H, H-6), 3.81 (dd, 1H, H-9b), 2.95 (m, 2H, 2OH), 2.26–2.14 (m, 2H, $2CH_{cyclohex.}$), 1.83–1.78 (m, 2H, $2CH_{cyclohex.}$), 1.63 (m, 3H, $3CH_{cyclohex.}$), 1.36–1.11 (m, 3H, $3CH_{cyclohex.}$); β pyran: δ 5.05 (br s, 1H, H-7), 4.17 (dd, 1H, $J_{9a,10}$=1.7 Hz, $J_{9a,9b}$=12.9 Hz, H-9a), 4.13 (m, 1H, H-6), 3.99 (dd, 1H, $J_{9b,10}$=3.6 Hz, H-10), 3.63 (dd, 1H, H-9b); α furan: δ 5.50 (d, 1H, $J_{8,9}$=5.1 Hz, H-8), 4.29 (d, 1H, H-9); β furan: δ 5.34 (d, 1H, $J_{8,OH}$=6.6 Hz, OH-8), 5.16 (m, 1H, H-8), 3.91 (m, 1H, H-1′a), 3.56 (m, 1H, H-1′b); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$): α pyran: δ 184.7 (C-2), 174.5 (C-4), 95.0 (C-7), 72.1 (C-6), 69.8 (C-5), 68.8 (C-10), 64.8 (C-9), 55.4 (C-1′), 29.2, 29.1, 26.7, 26.6, 25.9 ($5CH_2$); β pyran: δ 184.3 (C-2), 176.9 (C-4), 94.4 (C-7), 70.8 (C-5), 68.4 (C-10), 68.2 (C-6), 61.3 (C-9), 55.7 (C-1′), 29.1, 26.5, 25.9 ($3CH_2$); α furan: δ 100.8 (C-8); β furan: δ 92.6 (C-8), 59.3 (C-1′); LSIMS *m*/*z* 339 ([M+Na]$^+$, 29%); HRLSI-MS calcd for $C_{13}H_{20}N_2NaO_5S$ ([M+Na]$^+$): 339.0991, found: 339.0982.

*5.2.5. 5-O-tert-Butyldiphenylsilyl-3-deoxy-3-formamido-1,2-O-isopropylidene-3-C-methoxycarbonyl-β-D-arabinofuranose (**21**).* To a solution of aminoester **5** (102 mg, 0.21 mmol) in a 1:1 mixture of satd aq $NaHCO_3$–CH_2Cl_2 (5 mL) was added freshly-prepared AFA (86 μL, 0.64 mmol), and the mixture was vigorously stirred for 2.5 h. Then, the phases were separated and the aqueous phase was extracted with CH_2Cl_2 (2×10 mL); the combined organic fractions were dried over $MgSO_4$, filtered, and the filtrate was concentrated to dryness. The residue was purified by column chromatography (4:1→2:1 hexane–EtOAc) to give formamide **21** as a foam: 91 mg, 84%; R_f 0.29 (3:2 hexane–EtOAc); $[\alpha]_D^{23}$ −24 (*c* 0.83, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, 1H, $J_{NH,CHO}$=1.2 Hz, CHO), 7.61–7.56 (m, 4H, Ar–H), 7.46–7.40 (m, 6H, Ar–H), 6.82 (s, 1H, NH), 6.05 (d, 1H, $J_{1,2}$=3.9 Hz, H-1), 5.16 (d, 1H, H-2), 4.71 (dd, 1H, $J_{4,5a}$=6.6 Hz, $J_{4,5b}$=8.1 Hz, H-4), 4.00 (m, 2H, H-5a, H-5b), 3.76 (s, 3H, OMe), 1.50, 1.36 (2s, 3H each, $2CH_3$), 1.02 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 168.5 (CO), 160.2 (CHO) 135.6 (×2), 133.0, 132.8, 130.0, 127.3 (Ar–C), 116.7 (CMe_2), 104.9 (C-1), 88.0 (C-2), 80.5 (C-4), 68.5 (C-3), 63.1 (C-5), 52.6 (OMe), 27.7, 27.2 ($2CH_3$), 26.8 ($C(CH_3)_3$), 19.2 ($C(CH_3)_3$); CIMS *m*/*z* 414 ([M+H]+, 2%); HRCI-MS calcd for $C_{27}H_{36}NO_7Si$ ([M+H]$^+$): 514.2261, found: 514.2237.

*5.2.6. 5-O-tert-Butyldiphenylsilyl-3-deoxy-3-isoselenocyanato-1,2-O-isopropylidene-3-C-methoxycarbonyl-β-D-arabinofuranose (**23**).* To a mixture of formamide **21** (28 mg, 0.05 mmol), Et_3N (38.5 μL, 0.27 mmol) and 4 Å molecular sieves in anhydrous toluene (1 mL) was dropwise added a solution of triphosgene (13 mg, 0.04 mmol) at 0 °C and inert atmosphere during a period of 30 min. After that, the mixture was kept stirring at 0 °C for further 15 min, and heated at 90 °C for 3.5 h; a less polar compound was observed by TLC, corresponding to transient isocyanide. Then, black selenium (27 mg, 0.35 mmol) and Et_3N (7.6 μL, 0.05 mmol) were added, and the corresponding mixture was heated at 90 °C in the darkness for 36 h. After that, the reaction was diluted with CH_2Cl_2 (5 mL), the salts were filtered off on a Celite pad, and the filtrate was concentrated to dryness. The residue was purified by column chromatography (19:1 hexane–EtOAc) to give isoselenocyanate **23** as a foam: 12 mg, 39%; R_f 0.65 (4:1 hexane-EtOAc); $[\alpha]_D^{24}$ −92 (*c* 0.46, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67–7.63 (m, 4H, Ar–H), 7.44–7.36 (m, 6H, Ar–H), 5.86 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 4.86 (d, 1H, H-2), 4.27 (dd, 1H, $J_{4,5a}$=7.8 Hz, $J_{4,5b}$=6.0 Hz, H-4), 4.16 (dd, 1H, $J_{5a,5b}$=10.2 Hz, H-5a) 3.94 (dd, 1H, H-5b), 3.79 (s, 3H, OMe), 1.43, 1.34 (2s, 3H each, $2CH_3$), 1.05 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 163.5 (CO), 135.8, 135.7 (Ar–C), 134.3 (NCSe), 133.1, 132.9, 130.0 (×2), 128.0, 127.9 (Ar–C), 116.9 (CMe_2), 104.8 (C-1), 88.9 (C-2), 85.9 (C-4), 72.4 (C-3), 62.3 (C-5), 53.3 (OMe), 27.4 (×2) ($2CH_3$), 26.9 ($C(CH_3)_3$), 19.3 ($C(CH_3)_3$); LSIMS *m*/*z* 598 ([M+Na]$^+$, 7%); HRLSI-MS calcd for $C_{27}H_{33}NNaO_6SeSi$ ([M+Na]$^+$): 598.1140, found: 598.1126.

5.3. General procedure for the preparation of spiroselenohydantoins 26 and 27

To a solution of aminoester **5** (100 mg, 0.21 mmol) in THF (1.5 mL) was added *p*-tolyl or α-naphthyl isoselenocyanates

(0.31 mmol), and the mixture was stirred at inert atmosphere and in the darkness until disappearance of the starting material was observed by TLC. Then, crude reaction was concentrated to dryness, and the residue was purified by column chromatography, using the eluant indicated in each case.

*5.3.1. (5R,6S,8S,9S)-6-tert-Butyldiphenylsilyloxymethyl-8,9-(dimethylmethylenedioxy)-3-p-methylphenyl-2-selenoxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**26**).* *p*-Tolyl isoselenocyanate (60 mg) was used, and the reaction proceeded for 16 h. Crude reaction was purified by column chromatography (9:1 →4:1 hexane–EtOAc) to give selenohydantoin **26** as a foam: 102 mg, 75%; R_f 0.33 (4:1 hexane–EtOAc); $[\alpha]_D^{25}$ −52 (*c* 0.50, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.66–7.60 (m, 4H, Ar–H), 7.41–7.31 (m, 6H, Ar–H), 7.20 (m, 4H, Ar–H), 5.94 (d, 1H, $J_{8,9}$=3.6 Hz, H-8), 4.76 (d, 1H, H-9), 4.35–4.30 (m, 2H, H-6, H-1′a), 3.86 (m, 1H, H-1′b), 2.39 (s, 3H, PhC*H*$_3$), 1.49, 1.31 (2s, 3H each, $2CH_3$), 0.99 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 139.8, 135.7, 133.2, 133.1, 130.4, 130.2, 129.9, 127.9 (×3), 127.7 (Ar–C), 115.8 (*C*Me$_2$), 105.5 (C-8), 87.5 (C-9), 84.8 (C-6), 62.2 (C-1′), 27.2 (CH_3), 27.1 ($C(CH_3)_3$), 26.9 (CH_3), 21.4 (PhC*H*$_3$), 19.3 (*C*$(CH_3)_3$); LSIMS *m*/*z* 673 ($[M+Na]^+$, 21%); HRLSI-MS calcd for $C_{33}H_{38}N_2NaO_5^{80}SeSi$ ($[M+Na]^+$): 673.1613, found: 673.1613.

*5.3.2. (5R,6S,8S,9S)-6-tert-Butyldiphenylsilyloxymethyl-8,9-(dimethylmethylenedioxy)-3-α-naphthyl-2-selenoxo-7-oxa-1,3-diazaspiro[4.4]nonan-4-one (**27**).* α-Naphthyl isoselenocyanate (73 mg) was used, and the reaction proceeded for 40 h. Crude reaction was purified by column chromatography (9:1 →4:1 hexane–EtOAc) to give selenohydantoin **27** as a foam: 138 mg, 96%; R_f 0.25 (4:1 hexane–EtOAc); $[\alpha]_D^{24}$ −29 (*c* 0.44, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.23 (br s, 1H, NH), 8.01 (d, 1H, $J_{H,H}$=8.1 Hz, Ar–H), 7.91 (m, 1H, $J_{H,H}$=8.1 Hz, Ar–H), 7.68–7.21 (m, 15H, Ar–H), 5.87 (d, 1H, $J_{8,9}$=3.9 Hz, H-8), 4.97 (d, 1H, H-9), 4.45–4.35 (m, 2H, H-6, H-1′a), 3.90 (dd, 1H, $J_{6,1'b}$=3.0 Hz, $J_{1'a,1'b}$=9.2 Hz, H-1′b), 1.38, 1.30 (2s, 3H each, $2CH_3$), 0.97 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 184.0 (C-2), 166.7 (C-4), 135.7, 134.4, 133.0, 132.8, 130.8, 130.2, 130.1, 130.0, 129.9, 128.6, 128.0, 127.9, 127.7 (×2), 126.7, 125.4, 122.7 (Ar–C), 116.1 (*C*Me$_2$), 105.0 (C-8), 87.0 (C-9), 84.3 (C-6), 71.7 (C-5), 62.4 (C-1′), 27.0 ($C(CH_3)_3$), 27.0, 26.3 ($2CH_3$), 19.3 (*C*$(CH_3)_3$); LSIMS *m*/*z* 709 ($[M+Na]^+$, 17%); HRLSI-MS calcd for $C_{36}H_{38}N_2NaO_5^{80}SeSi$ ($[M+Na]^+$): 709.1613, found: 709.1633.

5.4. General procedure for the synthesis of triazoles 28–31

To a solution of azidoester **4** (110 mg, 0.21 mmol) in a 1:1 mixture of acetone–H_2O (8 mL) were added copper(II) sulfate (14.4 mg, 0.09 mmol), sodium ascorbate (31.2 mg, 0.16 mmol) and the corresponding alkyne. The mixture was stirred at rt during the time indicated in each case. Then, the mixture was concentrated to dryness, and the residue was dissolved in CH_2Cl_2 (15 mL) and washed with water (15 mL). The aqueous phase was extracted with CH_2Cl_2 (2×50 mL); the combined organic fractions were dried over $MgSO_4$, filtered and the filtrate was concentrated to dryness. The residue was purified by column chromatography, using the eluant indicated in each case to give title triazoles as foams.

*5.4.1. 5-O-tert-Butyldiphenylsilyl-3-deoxy-1,2-O-isopropylidene-3-C-methoxycarbonyl-3-(4′-phenyl-1′H-1′,2′,3′-triazol-1′-yl)-β-D-arabinofuranose (**28**).* Phenylacetylene (40 μL, 0.36 mmol) was used, and the reaction proceeded during 48 h. Column chromatography (19:1 →9:1 hexane–EtOAc) afforded triazole **28**: 80 mg, 62%; R_f 0.44 (4:1 hexane–EtOAc); $[\alpha]_D^{28}$ −13 (*c* 1.04, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (s, 1H, H-5′), 7.67–7.59 (m, 6H, Ar–H), 7.45–7.29 (m, 9H, Ar–H), 5.96 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 5.81 (d, 1H, H-2), 4.56 (t, 1H, $J_{4,5a}$=$J_{4,5b}$=6.3 Hz, H-4), 4.41 (dd, 1H, $J_{5a,5b}$=10.5 Hz, H-5a) 4.17 (dd, 1H, H-5b), 3.78 (s, 3H, OMe), 1.49, 1.39 (2s, 3H each, $2CH_3$), 1.06 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 165.0 (C=O), 148.0 (C-4′), 135.7, 135.6, 133.2, 133.0, 130.2, 130.1, 130.0, 128.9, 128.4, 128.0, 125.9 (Ar–C), 120.6 (C-5′), 115.9 (*C*Me$_2$), 104.9 (C-1), 86.8 (C-4), 86.3 (C-2), 75.7 (C-3), 63.5 (C-5), 53.2 (OMe), 27.1 (CH_3), 27.0 ($C(CH_3)_3$), 26.8 (CH_3), 19.5 (*C*$(CH_3)_3$); LSIMS *m*/*z* 636 ($[M+Na]^+$, 67%); HRLSI-MS calcd for $C_{34}H_{39}N_3NaO_6Si$ ($[M+Na]^+$): 636.2506, found: 636.2508.

*5.4.2. 5-O-tert-Butyldiphenylsilyl-3-[4′-(cyclohex-1″-en-1″-yl)-1′H-1′,2′,3′-triazol-1′-yl]-3-deoxy-1,2-O-isopropylidene-3-C-methoxycarbonyl-β-D-arabinofuranose (**29**).* 1-Ethynylcyclohexene (50 μL, 0.43 mmol) was used, and the reaction proceeded for 22 h. Column chromatography (9:1 hexane–EtOAc) afforded triazole **29**: 88 mg, 67%; R_f 0.50 (4:1 hexane–EtOAc); $[\alpha]_D^{28}$ −12 (*c* 1.08, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62–7.60 (m, 5H, Ar–H, H-5′), 7.45–7.33 (m, 6H, Ar–H), 6.45 (m, 1H, H-2″), 5.91 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 5.73 (d, 1H, H-2), 4.52 (t, 1H, $J_{4,5a}$=$J_{4,5b}$=6.3 Hz, H-4), 4.36 (dd, 1H, $J_{5a,5b}$=10.5 Hz, H-5a), 4.14 (dd, 1H, H-5b), 3.73 (s, 3H, OMe), 2.23–2.16 (m, 4H, $2CH_2$), 1.75–1.64 (m, 4H, $2CH_2$), 1.44, 1.36 (2s, 3H each, $2CH_3$), 1.06 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 165.0 (C=O), 149.7 (C-4′), 135.7, 135.6, 133.2, 133.1, 123.0, 129.2, 127.9 (×2), 127.0 (Ar-C, C-1″), 125.8 (C-2″), 118.9 (C-5′), 115.6 (*C*Me$_2$), 104.9 (C-1), 87.1 (C-4), 86.0 (C-2), 75.5 (C-3), 63.6 (C-5), 53.1 (OMe), 27.0 (*C*$(CH_3)_3$), 26.9, 26.7 ($2CH_3$), 26.3, 25.4, 22.5, 22.3 ($4CH_2$), 19.4 (*C*$(CH_3)_3$); LSIMS *m*/*z* 640 ($[M+Na]^+$, 16%); HRLSI-MS calcd for $C_{34}H_{43}N_3NaO_6Si$ ($[M+Na]^+$): 640.2819, found: 640.2842.

*5.4.3. 5-O-tert-Butyldiphenylsilyl-3-(4′-cyclopentyl-1′H-1′,2′,3′-triazol-1′-yl)-3-deoxy-1,2-O-isopropylidene-3-C-methoxycarbonyl-β-D-arabinofuranose (**30**).* Cyclopentylacetylene (49 μL, 0.42 mmol) was used, and the reaction proceeded for 12 h. Column chromatography (4:1 hexane–EtOAc) afforded triazole **30**: 108 mg, 86%; R_f 0.34 (4:1 hexane–EtOAc); $[\alpha]_D^{24}$ −7 (*c* 0.98, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62–7.60 (m, 4H, Ar–H), 7.55 (s, 1H, H-5′), 7.51–7.33 (m, 6H, Ar–H), 5.91 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 5.69 (d, 1H, H-2), 4.55 (t, 1H, $J_{4,5a}$=$J_{4,5b}$=6.3 Hz, H-4), 4.34 (dd, 1H, $J_{5a,5b}$=10.5 Hz, H-5a), 4.12 (dd, 1H, H-5b), 3.74 (s, 3H, OMe), 3.10 (q, 1H, $J_{1'',2''}$=$J_{1'',5''}$=8.1 Hz, H-1″), 2.04–2.00 (m, 2H, CH_2), 1.77–1.54 (m, 6H, $3CH_2$), 1.43, 1.36 (2s, 3H each, $2CH_3$), 1.05 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 165.2 (C=O), 152.8 (C-4′), 135.7, 135.6, 133.2, 133.1, 130.0, 129.9, 127.9 (×2), (Ar–C), 120.2 (C-5′), 115.5 (*C*Me$_2$), 104.9 (C-1), 87.0 (C-4), 86.1 (C-2), 75.5 (C-3), 63.6 (C-5), 53.0 (OMe), 36.8 (C-1″), 33.2, 33.1 ($2CH_2$), 27.0 ($C(CH_3)_3$), 26.9, 26.7 ($2CH_3$), 25.3 (×2) ($2CH_2$), 19.4 (*C*$(CH_3)_3$); CIMS *m*/*z* 606 ($[M+H]^+$, 60%); HRCI-MS calcd for $C_{33}H_{44}N_3O_6Si$ ($[M+H]^+$): 606.2999, found: 606.2978.

*5.4.4. 5-O-tert-Butyldiphenylsilyl-3-(4′-n-butyl-1′H-1′,2′,3′-triazol-1′-yl)-3-deoxy-1,2-O-isopropylidene-3-C-methoxycarbonyl-β-D-arabinofuranose (**31**).* Hex-1-yne (48 μL, 0.42 mmol) was used, and the reaction proceeded for 12 h. Column chromatography (9:1 hexane–EtOAc) afforded triazole *31*: 97 mg, 76%; R_f 0.52 (4:1 hexane–EtOAc); $[\alpha]_D^{23}$ −7 (*c* 1.14, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64–7.60 (m, 4H, Ar–H), 7.52 (s, 1H, H-5′), 7.45–7.34 (m, 6H, Ar–H), 5.91 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 5.71 (d, 1H, H-2), 4.54 (t, 1H, $J_{4,5a}$=$J_{4,5b}$=6.3 Hz, H-4), 4.35 (dd, 1H, $J_{5a,5b}$=10.5 Hz, H-5a), 4.13 (dd, 1H, H-5b), 3.73 (s, 3H, OMe), 2.65 (t, 2H, $J_{1'',2''}$=7.6 Hz, H-1″), 1.58 (m, 2H, H-2″), 1.43, 1.34 (2s, 3H each, $2CH_3$), 1.41–1.26 (m, 2H, H-3″), 1.05 (s, 9H, $C(CH_3)_3$), 0.91 (t, 3H, $J_{3'',4''}$=7.3 Hz, H-4″); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 165.1 (C=O), 148.6 (C-4′), 135.7, 135.6, 133.1, 130.0, 129.9, 127.9 (×2) (Ar–C), 121.3 (C-5′), 115.6 (*C*Me$_2$), 104.9 (C-1), 87.0 (C-4), 86.0 (C-2), 75.4 (C-3), 63.5 (C-5), 53.0 (OMe), 31.4 (C-1″), 26.9 ($C(CH_3)_3$), 26.9, 26.7 ($2CH_3$), 25.4 (C-2″), 22.5 (C-3″), 19.4 (*C*$(CH_3)_3$), 13.9 (C-4″); CIMS *m*/*z* 594 ($[M+H]^+$, 12%); HRCI-MS calcd for $C_{32}H_{44}N_3O_6Si$ ($[M+H]^+$): 594.2999, found: 594.3003; Anal.

Calcd for $C_{32}H_{43}N_3O_6Si$: C, 64.73; H, 7.30; N, 7.08, found: C, 64.78; H, 7.39; N, 7.06.

5.5. General method for the deprotection of triazoles 28–31

A solution of triazoles **28**–**31** (0.14 mmol) in a 9:1:2 mixture of TFA–H_2O–THF (12 mL) was stirred at 50 °C for 20 h. Then, the solvent was removed under reduced pressure and co-evaporated with toluene several times. The residue was purified as indicated in each case to give triazolyl lactones **32**–**35** as anomeric mixtures.

*5.5.1. 3-C-3,5-Carbolactono-3-deoxy-3-(4′-phenyl-1′H-1′,2′,3′-triazol-1′-yl)-D-arabinofuranose (**32**).* Triazole **28** was used. Crude reaction was washed with hexane (3×10 mL) to give triazole **32** as a foam: 36 mg (86%); R_f 0.14 (5:1 CH_2Cl_2–MeOH); $[\alpha]_D^{30}$ −92 (*c* 1.38, $(CH_3)_2CO$); ^1H NMR (300 MHz, $(CD_3)_2CO$) α anomer: δ 8.79 (s, 1H, H-5′), 7.96 (m, 2H, Ar–H), 7.48–7.42 (m, 2H, Ar–H), 7.38–7.32 (m, 1H, Ar–H), 5.85 (dd, 1H, $J_{4,5a}$=6.3 Hz, $J_{4,5b}$=2.7 Hz, H-4), 5.60 (s, 1H, $J_{1,2}\approx 0$ Hz, H-1), 5.16 (s, 1H, H-2), 4.85 (dd, 1H, $J_{5a,5b}$=10.2 Hz, H-5a), 4.45 (dd, 1H, H-5b); β anomer: δ 8.74 (s, 1H, H-5′), 7.96 (m, 2H, Ar–H), 7.48–7.42 (m, 2H, Ar–H), 7.38–7.32 (m, 1H, Ar–H), 5.56 (d, 1H, $J_{1,2}$=3.3 Hz, H-1), 5.52 (d, 1H, $J_{4,5a}$=5.1 Hz, $J_{4,5b}\approx 0$ Hz, H-4), 5.05 (d, 1H, H-2), 4.83 (dd, 1H, $J_{5a,5b}$=10.5 Hz, H-5a), 4.57 (d, 1H, H-5b); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$): δ 131.6, 131.5, 129.7, 129.0, 128.9, 126.4, 126.3 (Ar–C); α anomer: δ 170.1 (C=O), 148.1 (C-4′), 120.8 (C-5′), 105.6 (C-1), 83.1 (C-4), 82.1 (C-2), 77.9 (C-3), 72.6 (C-5); β anomer: δ 171.2 (C=O), 148.3 (C-4′), 121.7 (C-5′), 96.7 (C-1), 80.9 (C-4), 79.4 (C-2), 74.8 (C-5), 70.5 (C-3); LSIMS *m*/*z* 304 ($[M+H]^+$, 3%); 326 ($[M+Na]^+$, 6%); HRLSI-MS calcd for $C_{14}H_{14}N_3O_5$ ($[M+H]^+$): 304.0933, found: 304.0923.

*5.5.2. 3-C-3,5-Carbolactono-3-[4′-(cyclohex-1″-en-1″-yl)-1′H-1′,2′,3′-triazol-1′-yl]-3-deoxy-D-arabinofuranose (**33**).* Triazole **29** was used. Crude reaction was purified by gel permeation chromatography (Biogel P2) to give triazole **33** as a foam: 20 mg (50%); R_f 0.15 (5:1 CH_2Cl_2–MeOH); $[\alpha]_D^{24}$ −148 (*c* 0.80, $(CH_3)_2CO$); ^1H NMR (300 MHz, $(CD_3)_2CO$): α anomer: δ 8.25 (s, 1H, H-5′), 6.56–6.49 (m, 1H, H-2″), 5.77 (dd, 1H, $J_{4,5a}$=6.0 Hz, $J_{4,5b}$=2.7 Hz, H-4), 5.56 (s, 1H, $J_{1,2}\approx 0$ Hz, H-1), 5.07 (s, 1H, H-2), 4.80 (dd, 1H, $J_{5a,5b}$=10.2 Hz, H-5a), 4.41 (dd, 1H, H-5b), 2.43–2.37 (m, 2H, H-6″), 2.22–2.16 (m, 2H, H-3″), 1.80–1.71 (m, 2H, H-5″), 1.69–1.62 (m, 2H, H-4″); β anomer: δ 8.19 (s, 1H, H-5′), 6.56–6.49 (m, 1H, H-2″), 5.52 (d, 1H, $J_{1,2}$=3.9 Hz, H-1), 5.46 (dd, 1H, $J_{4,5a}$=5.4 Hz, $J_{4,5b}$=0.9 Hz, H-4), 4.93 (d, 1H, H-2), 4.79 (dd, 1H, $J_{5a,5b}$=10.5 Hz, H-5a), 4.52 (dd, 1H, H-5b), 2.43–2.37 (m, 2H, H-6″), 2.22–2.16 (m, 2H, H-3″), 1.80–1.71 (m, 2H, H-5″), 1.69–1.62 (m, 2H, H-4″); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$): α anomer: δ 170.2 (C=O), 149.8 (C-4′), 128.3 (C-1″), 125.5 (C-2″), 119.1 (C-5′), 105.7 (C-1), 83.3 (C-4), 82.2 (C-2), 77.7 (C-3), 72.5 (C-5), 26.8 (C-6″), 25.8 (C-3″), 23.1 (C-5″), 22.9 (C-4″); β anomer: δ 171.1 (C=O), 150.0 (C-4′), 128.2 (C-1″), 125.2 (C-2″), 120.1 (C-5′), 96.7 (C-1), 80.8 (C-4), 79.6 (C-2), 74.7 (C-5), 70.2 (C-3), 26.8 (C-6″), 25.8 (C-3″), 23.1 (C-5″), 22.9 (C-4″); CIMS *m*/*z* 308 ($[M+H]^+$, 52%); HRCI-MS calcd for $C_{14}H_{18}N_3O_5$ ($[M+H]^+$): 308.1246, found: 308.1245.

*5.5.3. 3-C-3,5-Carbolactono-3-(4′-cyclopentyl-1′H-1′,2′,3′-triazol-1′-yl)-3-deoxy-D-arabinofuranose (**34**).* Triazole **30** was used. Crude reaction was washed with Et_2O (10 mL) and CH_2Cl_2 (10 mL) to give triazole **34** as a foam: 28 mg (64%); R_f 0.15 (5:1 CH_2Cl_2–MeOH); $[\alpha]_D^{26}$ −128 (*c* 0.93, DMSO); ^1H NMR (300 MHz, DMSO-d_6): α anomer: δ 8.25 (s, 1H, H-5′), 6.83 (d, 1H, $J_{OH,1}$=3.7 Hz, OH-1), 6.57 (d, 1H, $J_{OH,2}$=5.0 Hz, OH-2), 5.56 (dd, 1H, $J_{4,5a}$=6.3 Hz, $J_{4,5b}$=2.6 Hz, H-4), 5.37 (d, 1H, $J_{1,2}\approx 0$ Hz, H-1), 4.94 (d, 1H, H-2), 4.75 (dd, 1H, $J_{5a,5b}$=10.1 Hz, H-5a), 4.33 (dd, 1H, H-5b), 3.18–3.10 (m, 1H, H-1″), 2.08–1.95 (m, 2H, CH_2), 1.71–1.56 (m, 6H, $3CH_2$); β anomer: δ 8.16 (s, 1H, H-5′), 7.23 (d, 1H, $J_{OH,1}$=4.0 Hz, OH-1), 5.71 (d, 1H, $J_{OH,2}$=8.0 Hz, OH-2), 5.33 (t, 1H, $J_{1,2}$=3.7 Hz, H-1), 5.24 (d, 1H, $J_{4,5a}$=5.8 Hz, $J_{4,5b}\approx 0$ Hz, H-4), 4.85 (dd, 1H, H-2), 4.68 (dd, 1H, $J_{5a,5b}$=10.4 Hz, H-5a), 4.40 (d, 1H, H-5b), 3.18–3.10 (m, 1H, H-1″), 2.08–1.95 (m, 2H, CH_2), 1.71–1.56 (m, 6H, $3CH_2$); ^{13}C NMR (75.5 MHz, DMSO-d_6): α anomer: δ 169.6 (C=O), 151.3 (C-4′), 121.1 (C-5′), 104.2 (C-1), 81.8 (C-4), 79.9 (C-2), 76.6 (C-3), 71.5 (C-5), 36.1 (C-1″), 32.7 (×2) ($2CH_2$), 24.7 ($2CH_2$); β anomer: δ 169.8 (C=O), 151.5 (C-4′), 120.1 (C-5′), 95.9 (C-1), 79.7 (C-4), 77.6 (C-2), 73.4 (C-5), 70.0 (C-3), 36.1 (C-1″), 32.7 (×2) ($2CH_2$), 24.7 ($2CH_2$); CIMS *m*/*z* 296 ($[M+H]^+$, 50%); HRCI-MS calcd for $C_{13}H_{18}N_3O_5$ ($[M+H]^+$): 296.1246, found: 296.1247.

*5.5.4. 3-(4′-n-Butyl-1′H-1′,2′,3′-triazol-1′-yl)-3-C-3,5-carbolactono-3-deoxy-D-arabinofuranose (**35**).* Triazole **31** was used. Crude reaction was purified by gel permeation chromatography (Biogel P2) to give triazole **35** as a foam: 14 mg (36%); R_f 0.16 (5:1 CH_2Cl_2–MeOH); $[\alpha]_D^{23}$ −180 (*c* 0.93, $(CH_3)_2CO$); ^1H NMR (300 MHz, $(CD_3)_2CO$): α anomer: δ 8.07 (s, 1H, H-5′), 5.74 (dd, 1H, $J_{4,5a}$=6.3 Hz, $J_{4,5b}$=2.7 Hz, H-4), 5.56 (s, 1H, $J_{1,2}\approx 0$ Hz, H-1), 5.03 (s, 1H, H-2), 4.79 (dd, 1H, $J_{5a,5b}$=10.2 Hz, H-5a), 4.41 (dd, 1H, H-5b), 2.72 (t, 2H, $J_{1″,2″}$=7.5 Hz, H-1″), 1.71–1.60 (m, 2H, H-3″), 1.45–1.32 (m, 2H, H-2″), 0.93 (t, 2H, $J_{3″,4″}$=7.2 Hz, H-4″); β anomer: δ 8.06 (s, 1H, H-5′), 5.52 (d, 1H, $J_{1,2}$=4.2 Hz, H-1), 5.46 (dd, 1H, $J_{4,5a}$=5.4 Hz, $J_{4,5b}$=0.9 Hz, H-4), 4.89 (d, 1H, H-2), 4.79 (dd, 1H, $J_{5a,5b}$=10.5 Hz, H-5a), 4.72 (dd, 1H, H-5b), 2.70 (t, 2H, $J_{1″,2″}$=7.5 Hz, H-1″), 1.71–1.60 (m, 2H, H-2″), 1.45–1.32 (m, 2H, H-3″), 0.92 (t, 2H, $J_{3″,4″}$=7.2 Hz, H-4″); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$): α anomer: δ 170.1 (C=O), 148.6 (C-4′), 122.2 (C-5′), 105.6 (C-1), 83.3 (C-4), 82.2 (C-2), 77.5 (C-3), 72.4 (C-5), 32.2 (C-1″), 25.8 (CH_2), 22.8 (CH_2), 14.0 (CH_3); β anomer: δ 171.2 (C=O), 148.9 (C-4′), 122.4 (C-5′), 96.5 (C-1), 80.8 (C-4), 79.6 (C-2), 74.7 (C-5), 69.9 (C-3), 32.2 (C-1″), 25.8 (CH_2), 22.8 (CH_2), 14.0 (CH_3); CIMS *m*/*z* 284 ($[M+H]^+$, 28%); HRCI-MS calcd for $C_{12}H_{18}N_3O_5$ ($[M+H]^+$): 284.1246, found: 284.1252; Anal. Calcd for $C_{12}H_{17}N_3O_5$: C, 50.88; H, 6.05; N, 14.83, found: C, 50.61; H, 6.02; N, 15.01.

5.6. Enzymatic inhibition

The enzymatic assays were carried out as described previously.[42] 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, *p*-nitrophenyl-α-D-galactopyranoside, 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[43] by using commercial glycogen phosphorylase b (rabbit muscle, 10 mg/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,[44] using as developer 0.18 mM $FeSO_4$ and 0.05 M $(NH_4)MoO_4$ in the presence of 0.5 M H_2SO_4 at 720 nm. Hanes plots were built using the initial velocity from each reaction, as indicated above for glycosidases.

5.7. Free radical scavenging

The free radical-scavenging activity was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl radical) method[45] in a UV spectrometer using PS cuvettes.

To a 60 μM solution of DPPH in HPLC-grade methanol (1.7 mL) was added 30 μL of the antioxidant solution in MeOH (five different concentrations) or pure methanol (control). The corresponding mixture was kept in the darkness for 30 min and then the absorbance at 515 nm was measured against a MeOH blank in order to calculate the EC_{50} values, that is, the concentration of the antioxidant (expressed in μM) required to reduce the concentration of the DPPH to 50% of its initial value. All the measurements were carried out in triplicate. The remaining DPPH concentration was calculated using the expression:

$$\%\text{DPPH remaining} = \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{sample} and A_{control} refer to the absorbances at 515 nm of the free radical in the sample and control solutions, respectively.

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Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.tet.2012.03.087.

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