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A bioinspired look at the glucosinolate metabolic pathway. Structural insights into the reaction of benzyl isothiocyanate and D-glucosamine

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ABSTRACT

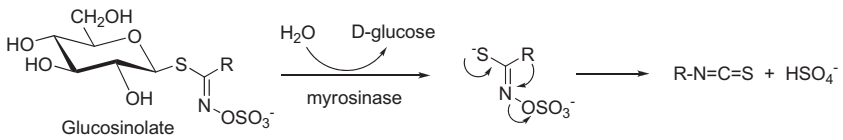
Through a well-established enzymatic transformation glucosinolates release reactive isothiocyanates that can undergo further metabolic pathways affording a plethora of reactive metabolites. This study explores in detail the reaction of benzyl isothiocyanate, which possesses antitumor activity as alkylating agent, with D-glucosamine, commonly employed in oral treatments against osteoarthritis and inflammation. Structures of the resulting products and their evolution have been assessed and compared with those involving D-glucose, reported previously. Chemical results suggest that clinical treatments with D-glucosamine could reduce the beneficial effects associated with diets based on glucosinolate-rich foods.

1. Introduction and background

Glucosinolates are a family of glycosides, which are enzymatically hydrolyzed in damaged plant tissues giving rise to potentially toxic metabolites. They are present in seeds of Cruciferae and Brassicaceae plants. Hydrolysis of the glucoside bond via the enzyme myrosinase, a thioglucosidase, results in a thiohydroximate sulfonate. This compound usually yields an isothiocyanate by a Lössen-type rearrangement prompted by the sulfate leaving group.[1]

The isothiocyanates (ITCs) are therefore an important group of breakdown products derived from glucosinolates (Scheme 1) and constitute powerful electrophiles capable of reacting, under physiological conditions, with a wide range of nucleophilic species, such as amino acids and peptides, and other biogenic amines, alcohols, and water. The degradation pathway induced by myrosinase is also influenced by factors, such as pH and the presence of metal ions.[2]

Epidemiological studies show that consumption of *Brassica* vegetables is associated with a reduced incidence of cancers at a number of sites including the lung, stomach, colon, and rectum.[3] The processes of ingestion and digestion of *Brassica* vegetables lead to glucosinolate hydrolysis. The liberated ITCs have shown to be potent and selective inhibitors of carcinogenesis induced by a variety of chemical carcinogens,[4] such as tobacco-derived nitrosamines and polycyclic aromatic hydrocarbons and appear to act at a number of stages during the tumor development process.[5] ITCs act as blocking agents by modifying the metabolism of carcinogenic compounds through their influence on biotransformation enzymes. The action of isothiocyanates is generally believed to enhance the activity of phase II enzymes,[6] with some evidence that they also



Scheme 1.

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inhibit phase I enzymes.[7] This dual action is thought to reduce the production of electrophilic intermediates with carcinogenic activity and to enhance the detoxification and clearance of carcinogens. ITCs also serve as suppressing agents during the promotion phase of the neoplastic process. Recent work has shown that ITCs act on signal transduction pathways within the cell, both inducing apoptosis and inhibiting cell growth.[8,9] In vivo, ITCs are conjugated with glutathione and then sequentially metabolized to mercapturic acids.[10]

One of the most intensively studied ITCs with regard to cancer chemoprevention and genotoxic effects is benzyl isothiocyanate (BITC), a product of enzymatic hydrolysis of glucotropaeolin.[11–13] BITC and its *m*-methoxy derivative appear to be the most abundant degradation products resulting from glucosinolates present in certain plants, such as Peruvian maca (*Lepidium meyenii*).[13]

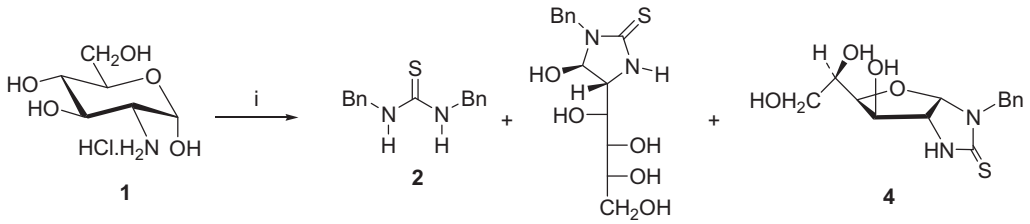
In a recent investigation, Kruse et al. have identified the main products resulting from the reaction of BITC with D-glucose.[14] Such substances were heterocycles derived from 1,3-oxazolidin-2-thiones and 1,3-thiazolidin-2-thione. Although a mechanistic study was lacking, the authors were unable to detect the presence of simple addition products like thiourethanes. Given our past experience on amino sugars and sugar isothiocyanates, the above research prompted us to evaluate the corresponding reaction of BITC with D-glucosamine, which could be an appropriate in vitro model on the interaction of secondary plant metabolites and nutrients or bioactive substances.

D-Glucosamine is a key precursor of peptidoglycan structures and plays therefore an important role in the production of collagenous fibers present in connective tissue, such as cartilages and synovial fluid.[15] D-Glucosamine is orally administered against bone degenerative diseases, especially osteoarthritis.[16] It also exhibits anti-inflammatory properties by inhibiting proteolytic enzymes; and in this context D-glucosamine in the form of food supplements represents a valuable surrogate of conventional agents like ibuprofen in reducing pain.[17] About 90% of D-glucosamine sulfate administered orally can be absorbed by the large intestine and crosses easily other biological barriers. Pharmacokinetic studies show variable levels of this and other amino sugars in serum after administration of chondroitin sulfate or glucosamine.[18]

Since both ITCs and D-glucosamine can be present in biological fluids, the present study explores structural and mechanistic details on their interaction under physiological-like conditions. Our study sheds also light into putative metabolic pathways and the product distribution is compared with that found previously with D-glucose.[14]

2. Results and discussion

The condensation of 2-amino-2-deoxy-D-glucopyranose (**1**) with BITC has been explored in an aqueous environment. At room temperature this reaction proceeds slowly and BITC is poorly soluble; only one new product having R_f 0.47 (CH_2Cl_2–MeOH, 4:1 v/v) could be detected. In order to speed up the transformation, both reactants were heated at 75 °C in a hydroalcoholic solution for 2 h (Scheme 2). The process took place smoothly and TLC monitoring revealed the formation of three major products (with 99% overall yield). One of them could easily be isolated on cooling and identified as *N*,*N*′-dibenzylthiourea (**2**, 37%).[19] Further inspection of the mother liquors showed the presence of two additional substances (R_f values: 0.47 and 0.62), which could be isolated by flash chromatography. Their elemental analyses and spectroscopic data are consistent with structures of (4*R*,5*S*)-1-benzyl-5-hydroxy-4-(D-*arabino*-tetritol-1-yl)-1,3-imidazolidin-2-thione (**3**, 27%) and 1-benzyl-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidin-2-thione (**4**, 35%), respectively.

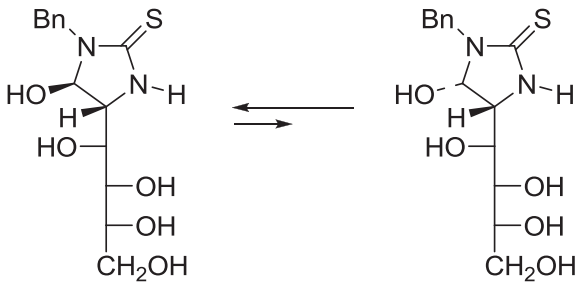


1 **2** **3** **4**

Scheme 2. i, $NaHCO_3$, $PhCH_2NCS$, Δ.

The structure attributed to **3** agrees with a molecular peak (*m*/*z* 328) determined by mass spectrometry. The existence of water in the crystal lattice is also evidenced by a weak and broad IR absorption at ~1638 cm^{-1}.[20] NMR spectra of **3** are similar to those of analogous derivatives.[21,22] The ^1H NMR spectrum shows signals for the NH and OH groups at the heterocyclic ring at 8.00 and 6.65 ppm, respectively, while ^{13}C NMR resonances for the thiocarbonyl group and C-5 appear at 181.0 and 83.7 ppm, respectively (Figs. S1–S3). The latter rules out an alternative isomeric structure of 2-benzylthioureido-2-deoxy-D-glucopiranose, for which the anomeric carbon would resonate at ~90 ppm (α anomer) or ~95 ppm (in the case of the β anomer).[21,23,24] The proton linked to C-5 (δ 5.31 ppm) exhibits a small coupling constant with the H-4 proton ($J_{4,5}$=3.2 Hz), thereby pointing to a trans relationship between such protons and, as a result the configuration at C-5 should be *R*.[21–23] The carbon atoms located along the polyhydroxyl side chain show chemical shifts similar to those found in acyclic fragments of sugar derivatives.[24]

Compound **3** equilibrated in solution with another substance exhibiting close NMR patterns and chemical shifts (Scheme 3). The latter was identified as the cis epimer (**5**), although the former was

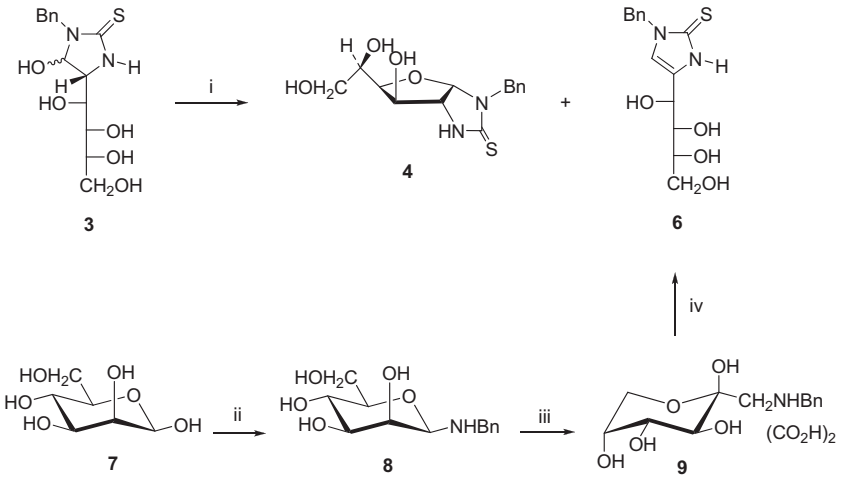


3 **5**

Scheme 3.

prevalent in the equilibrium (**3**: ~82%). The relative cis disposition between H-4 and H-5 in **5** could be inferred from the large coupling constant ($J_{4,5}$=7.6 Hz).[21–23] Similar epimerizations have been previously observed.[22a,25]

heating a solution of **3** in 30% aqueous acetic acid the same mixture of **4** and **6** was obtained in 67% overall yield (Scheme 4).



Scheme 4. i, AcOH 30%, Δ; ii, $BnNH_2$, EtOH, Δ; iii, $(CO_2H)_2$, Δ; iv, KSCN, Δ.

In both stereoisomers the benzyl protons appear as an AB system, thus pointing to their diastereotopic nature and resonating at very different chemical shifts ($\Delta\delta$ ~0.75 ppm, J_{gem}=16.0 Hz for **3**; $\Delta\delta$ ~1.07 ppm, J_{gem}=15.4 Hz for **5**). This strong anisotropy is not only due to the presence of a vicinal C=S group, but also to conformational restriction of the benzyl group.[26]

On the other hand, the mass spectrum of **4** shows a molecular peak at *m*/*z* 310. IR absorptions characteristic of the aromatic ring and OH groups are likewise consistent with its structure. Again, the presence of water in the crystal lattice is evidenced by a weak and broad absorption at ~1630 cm^{-1}, overlapping the thioamide NH band at ~1556 cm^{-1}.[20] The signals of the NH and the heterocyclic H-1 protons resonate at 8.90 and 5.59 ppm, whereas the thiocarbonyl and C-1 carbons appear at 182.8 and 92.8 ppm, respectively, and are close to those found in analogous bicyclic systems.[21–23b,27] A null coupling between H-2 and H-3 ($J_{2,3}$=0 Hz) along with the downfield chemical shift of C-4 (80.3 ppm), more deshielded than C-5 (68.7 ppm), suggest a furanoid structure for the sugar ring, while the large coupling constant $J_{1,2}$ (~6.6 Hz) is also characteristic of a cis fusion between the heterocyclic moiety and furanose rings (Figs. S4–S6).[21–23,27,28]

The fragmentation pattern observed in the mass spectrum of **4** also agree with the proposed structure for this substance (Scheme S1). Together with the above-mentioned molecular peak (*m*/*z* 310), characteristic peaks evidence the loss of one or two water molecules (*m*/*z* 292 and 274). Likewise, the peak at *m*/*z* 190 corresponds to the heterocyclic fragment, while the base peak can be attributed to a benzyl-tropilium ion (*m*/*z* 91).[29a] The mass spectrum of **3** shows its molecular peak (*m*/*z* 328) with low intensity, whilst the remaining signals are almost coincidental with those of **4**, which reveals that **3** losses one water molecule to give **4** and the latter undergoes the subsequent fragmentations.

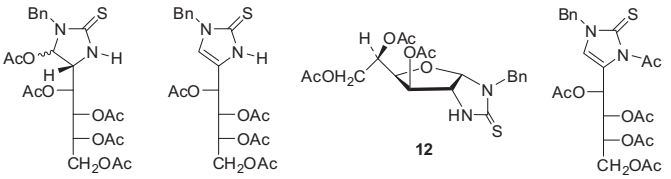
According to previous studies, the acid treatment of **3** would afford compound **4** by cyclization and water elimination.[21,22b,27] Thus, the condensation of **1** with BITC, adding acetic acid to the reaction mixture, resulted in formation of **4** plus the isomeric imidazoline-2-thione **6**. This process was rather sluggish and both substances could be isolated in low yields. In stark contrast, on

The structure of **6** could also be established by analytical and spectroscopic methods. The IR sharp absorption at ~1633 cm^{-1} is consistent with the endocyclic double bond. The heterocyclic moiety exhibits characteristic NMR signals for the NH (δ 11.92 ppm) and H-5 (δ 6.84 ppm) protons, which are strongly deshielded, whereas the thiocarbonyl group (δ 161.73 ppm) is shifted upfield relative to that of compound **3**. Furthermore, the chemical shifts for C-4 (δ 132.07 ppm) and C-5 (δ 115.91 ppm) are characteristic of olefinic carbons.[21,22] It is worth mentioning that the previously observed separation in the benzyl protons has essentially vanished and the former AB system almost collapses into a singlet ($\Delta\delta$ ~0.05 ppm, J_{gem}=14.8 Hz). This reduction in anisotropy should be ascribed to a greater conformational freedom because there is no substituent at C-5 (Figs. S7 and S8).

The low-resolution mass spectrum of **6** reveals a molecular peak at *m*/*z* 310 and $[M+Na]^+$ at *m*/*z* 333. Intense peaks can also be attributed to $[M+H]^+$ at *m*/*z* 311, and successive water elimination. An important fragmentation corresponds to loss of the phenyl group resulting in ions with *m*/*z* 233 (100%) and *m*/*z* 234 (Scheme S2).

Moreover, the imidazoline derivative **6** could be prepared by an alternative route as depicted in Scheme 4. Thus, D-mannose (**7**) was first converted into *N*-benzylfructosamine oxalate **9**,[30] through the intermediacy of *N*-benzylmannosylamine **8**, which was further treated with potassium thiocyanate.[31] The resulting product had the same physical and spectroscopic properties as the compound obtained in dehydratation reaction of **3**.

To further confirm the above structures, their acetylated derivatives were generated by conventional treatment with acetic anhydride and pyridine at −20 °C. The acetylation of **3** led to a reaction mixture, whose NMR analysis identified the presence of **10** and **11**, which could not be purified due to their facile decomposition. On the contrary, the acetylation of **4** afforded compound **12** in good yield. The latter showed its molecular peak at *m*/*z* 436, with intense fragmentations corresponding to ions $[M+H]^+$ (100%), $[M]^+$, and tropilium. Other diagnostic fragmentations derived from successive elimination of three molecules of acetic acid, as well as of acetic anhydride and acetyl, which are characteristic of polyacetylated sugars (Scheme S3).[29b]

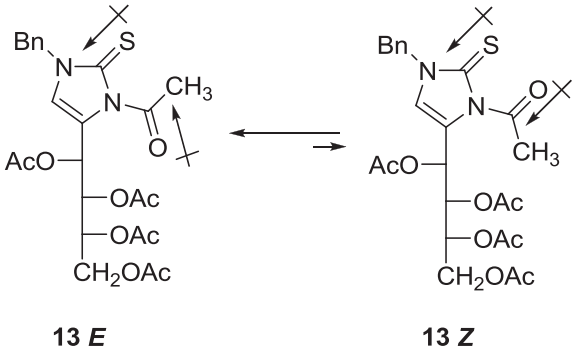


The absence of acetylation at the nitrogen atom of **12** under such mild acetylating conditions is evidenced by the IR absorption (NH stretching) at 3383 cm^{-1} and a proton signal at 6.50 ppm. In addition, there are only three signals for acetyl groups, and coupling constant values for $J_{4,5}$ (6.4 Hz) and $J_{4,1'}$ (0.0 Hz) are again consistent with both a furanoid structure for the sugar moiety and cis fusion between the two heterocycles (Figs. S9 and S10).

The acetylation of **6** gave rise to a mixture of **11** and **13** in variable ratios depending on the experimental conditions. Thus, acetylation at −20 °C resulted in the preferential formation of **11**; while compound **13** was instead prevalent at 80 °C. The monothioimide structure present in **13** enables a facile elimination of the *N*-acetyl group by solvolysis and, after crystallization from ethanol, **11** could be obtained as an analytically pure sample.

The low-resolution mass spectrum of **11** gave a molecular peak at *m*/*z* 478 (Scheme S4). The presence of the imidazolin-2-thione ring manifests itself by proton shifts of the NH and endocyclic =CH fragments at 11.22 ppm and 6.58 ppm, respectively, as well as the resonances of olefinic carbons: C-4 (124.1 ppm) and C-5 (116.3 ppm). In addition, four signals attributed to acetyl groups corroborate the identity of **11** still further (Figs. S11–S13).

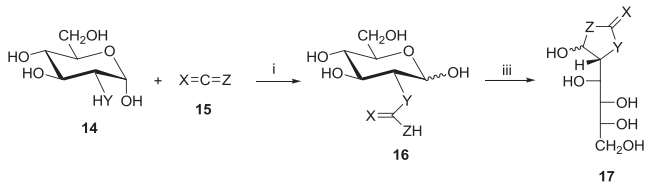
Compound **13** shows, in its ^1H NMR spectrum, five signals for the acetyl groups. Three of them resonate at ~2.1 ppm, which can be attributed to acetates, while two signals are shifted downfield (δ 3.14 ppm) and upfield (δ 1.66 ppm), respectively. The former does correspond to the acetyl group on the heterocyclic nitrogen; its unusual shift results from a preferential *E* conformation adopted by the acetamido group. This is the most stable conformation that minimizes the dipole effects caused by the carbonyl and thiocarbonyl groups (Scheme 5). In such a conformation the methyl group lies in the deshielding zone of the C=S bond.[21,27]



Scheme 5. Dipole effects in *E* and *Z* conformations of the acetamido group in **13**.

The *N*-acetyl group in compound **13** causes significant proton shift variations with respect to those found in **11**. While H-4 undergoes a slight shielding relative to its homologous signal in **11** ($\Delta\delta$ ~0.1 ppm), H-1′ is strongly deshielded ($\Delta\delta$ ~0.5 ppm). Moreover, the acetyl group at C-1′ lies in the shielding zone of the olefinic bond, thus undergoing a rather unusual chemical shift (δ ~1.66 ppm) for an acetyl group of a polyhydroxylated chain (~2.1 ppm) (Figs. S14 and S15).

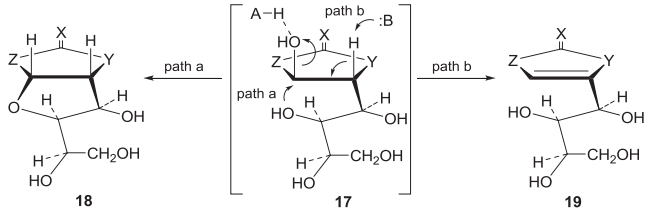
The reaction of monosaccharides and their amino derivatives (**14**) with heterocumulenes (**15**) has long been known and the mechanistic pathway is now well established (Scheme 6).[21–23] Such transformations occur at pH>7 via the addition of a hydroxyl (**14**, Y=O) or amino (**14**, Y=NR) group to the heterocumulenic system, followed by intramolecular cyclization of a transient intermediate (**16**) to give a heterocyclic derivative (**17**).



Scheme 6. The reaction of sugar and amino sugars with heterocumulenes.

Exhaustive studies have been conducted on 2-amino-2-deoxyaldoses (**14**, Y=NH or NR). In some circumstances, the intermediate **16** is stable enough and can be isolated, such as ureas (**16**, Y=NH, X=O, Z=NAr) arising from reaction of 2-amino-2-deoxyaldoses with aryl isocyanates.[23] The corresponding thioureas, however, are much more reactive and the final product **17** (Y=NH, X=S, Z=NAr) is generally the first isolable substance.[21]

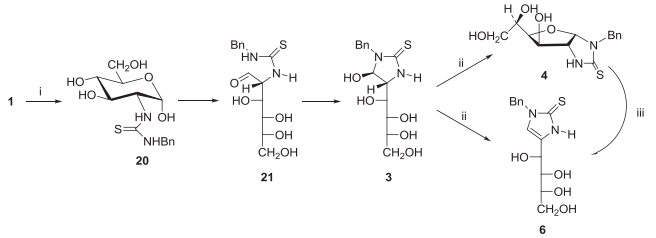
At pH<7 the monocyclic structures **17** can be converted into bicyclic ones (**18**) or unsaturated heterocycles (**19**). Both processes are indeed competitive and can be rationalized as depicted in Scheme 7; either a displacement reaction (path a) leading to an intramolecular cyclization, or an elimination reaction (path b) giving rise to an unsaturated derivative.[21–23]



Scheme 7.

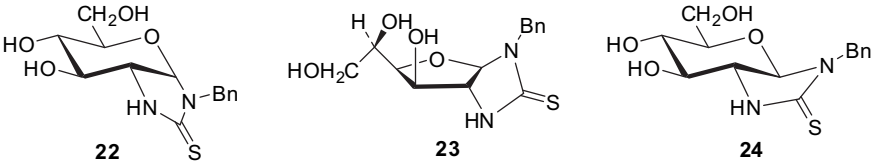
It is worth pointing out that the corresponding reaction involving amino sugars and carbon disulfide only produces the monocyclic structure (**17**, Y=NH, X=Z=S) without any trend to follow the above-mentioned transformations.[32,33] In contrast, similar structures to those obtained in the reactions of amino sugars with iso(thio)cyanates, i.e., **18** and/or **19**, have also been reported with potassium cyanate,[34,35] potassium thiocyanate,[36] and cyanamide.[37]

Given previous premises, the fate of the condensation of D-glucosamine (**1**) and BITC is collected in Scheme 8, which accounts for a rationale capable of explaining the product distribution observed. The process yields initially a thioureido derivative **20**, which via an aldehyde intermediate (**21**), cyclizes to the imidazolidin-2-thione **3**. Finally, under acid catalysis, the latter converts into a furanoid structure (**4**) or undergoes dehydration to yield **6**. Furthermore, the acid treatment of **4** may also cause its transformation into **6**.[38]



Scheme 8. i, BnNCS, pH>7; ii, AcOH 30%, Δ; iii, CF_3COOH, Δ.

This mechanism equally accounts for the regioselectivity and stereochemistry observed during the cyclization pathway, i.e., formation of a *cis*-fused furanoid bicycle (**4**). The Baldwin rules suggest that a *5-exo-tet* cyclization (**18**) should be entropically favored over a *6-exo-tet* ring closure leading to a *cis*-fused heterocycle,[39] which is in turn both the thermodynamic and kinetic product. Formation of alternative *cis*-fused bicycles having a pyranoid subunit, such as **22** or *trans*-fused structures like **23** and **24** have not yet been detected. The willingness to reach the most stable furanoid structure can even be observed under solvent-free ionizing conditions, such as in mass spectra recording. It should be mentioned, however, that analogous compounds of **22** and **24** have been synthesized.[21,40] In any case, *cis*-fused analogs of **23** have not been reported, due presumably to the greater steric tension associated with such a structure.

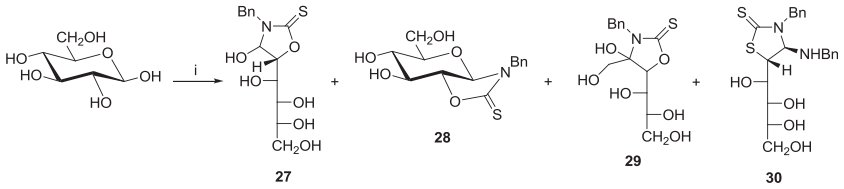


Although the reaction of monosaccharides or their 2-amino derivatives with heterocumulenes follows a well-established mechanism, some erroneous structures have appeared in the literature on describing related processes. Thus, it has been reported that the acid-catalyzed condensation of D-glucose and urea produces the bicyclic pyranose **25**;[41] although a further revision by the authors unveiled the most plausible structure **26**.[42]

In their recent study Kruse et al. reported that structures **27**–**30** should be associated to the four major products generated in the reaction of D-glucose and BITC (Scheme 9).[14] It is obvious that **27** represents the oxo-analogous derivative of **3**, from which a *cis*-configured bicyclic structure, such as **32**, and not the *trans*-configured one **28**, would result.

reaction with BITC, D-glucose had undergone the so-called Lobry de Bruyn–Alberda van Ekenstein isomerization,[47,48] via successive enolizations under the experimental basic conditions. Such isomerizations have in fact been reported in the reaction of D-glucosamine with aryl isocyanates at pH>10, to give 5-hydroxyimidazolidin-2-ones derived from D-mannosamine.[23a]

Once again, the coupling pattern to be expected for such substances would be markedly different to those measured experimentally for **32**. Thus, the $J_{2,3}$ values shown by **43** and **44** with D-*manno* configuration, which are the acetylated derivatives of **41** and **42**, respectively, and imidazolidin-2-ones **45** and **46**[23a] are much larger than zero (Table 1).[49]



Scheme 9. i, BnNCS, NaOH, pH ~11, Δ (95 °C).

The different bicyclic structures possessing D-*gluco* configuration can easily be assessed through an analysis of their coupling constants, which depend on the dihedral angle between vicinal protons. Table 1 shows these diagnostic values for compounds **4**, **25**,[40] **26**,[43] **32**,[14] **33**,[44] **34**,[27] **35**,[45] and **36**.[21]

A *cis*-fused furanoid structure present in compounds **4**, **26**, **32**–**34** manifests itself by a null or negligible $J_{2,3}$ coupling along with a medium-high value of $J_{1,2}$. Such couplings reflect directly the geometry adopted by such protons: an angle of ~25° between H-1 and H-2 placed in a relative cis disposition (**37**) and an angle of ~90° between H-2 and H-3 arranged in *trans* (**38**).

For pyranoid structures involving a trans fusion, like in **35**, the D-glucopyranose preserves its $^4C_1$ conformation, and hence all couplings show high values consistent with an antiperiplanar relationship for H-1, H-2, H-3, H-4, and H-5. In stark contrast, a cis fusion in the bicyclic framework, like in **25** and **36**, forces to a $^0S_2$ conformation, which results in moderate values for $J_{2,3}$ and $J_{3,4}$. The coupling $J_{1,2}$ is however high because the H-1 and H-2 protons are eclipsed (dihedral angle ~0°).[46] In addition, this conformation is also characterized by a W arrangement between H-2 and H-4, which translates into a significant long-range coupling $J_{2,4}$ (~0.8–1.3 Hz).[21]

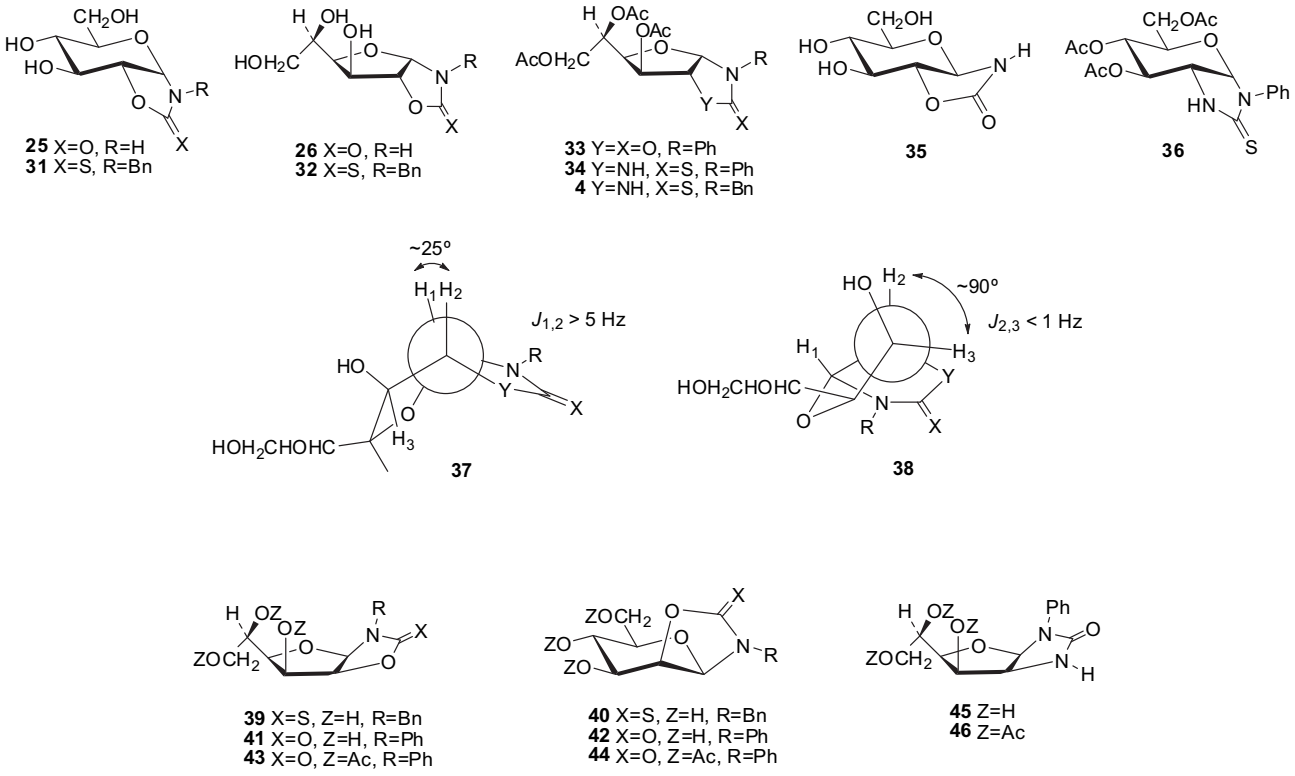
Accordingly, the couplings described for compounds **28** (or **31**) are actually consistent with the alternative structure **32**. The pyranoid structure for **28** (or **31**) was suggested on the basis of its mass-fragmentation pattern: the loss of 61 units from $[M+Na]^+$ to afford the peak of *m*/*z* 273.[14] Nevertheless, this fragmentation is also typical of furanoid structures, such as **32** and proceeds analogously to that of **4**, which is broken down to *m*/*z* 249.

Likewise, structures possessing D-*manno* configuration, both β-furanose (**39**) and β-pyranose (**40**), should be ruled out. As held previously,[14] these structures would have been formed if, prior to

Table 1
Coupling constants (Hz) for representative model compounds[a]

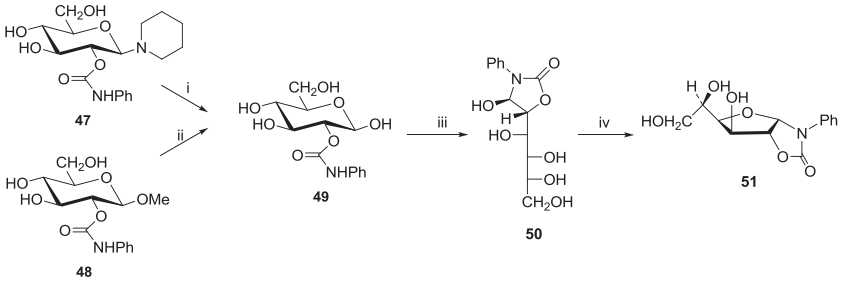
Compound	Structure	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
26	α-Furanose	5.4	0.6	2.4	9.0	2.5	5.6	12.2
32[b]		5.6	0.0	2.6	7.7	3.5	5.6	11.5
33		5.5	<0.5	2.8	9.1	2.4	5.2	12.3
34		6.3	0.0	1.8	8.6	2.3	5.4	11.0
4		6.4	0.0	2.8	9.2	2.4	5.6	12.2
35	β-Pyranose	8.8	10.8	7.7	9.7	2.1	5.5	12.4
25	α-Pyranose	6.5	4.7	4.7	—	2.2	3.4	12.4
36[c]		7.9	3.1	3.1	9.1	6.2	3.0	—
43	β-Furanose	6.2	5.7	4.0	9.0	2.5	5.0	12.1
44	β-Pyranose	3.7	3.5	—	6.0	3.0	5.0	12.3
45	β-Furanose	7.3	6.5	3.7	—	2.2	4.9	11.1
46	β-Furanose	7.3	6.5	4.2	8.9	2.5	4.9	12.3

[a] For comparative purposes, the numbering of the parent D-glucose has been maintained.
[b] Described as **28** or **31**.[14]
[c] $J_{2,4}$=0.8 Hz.

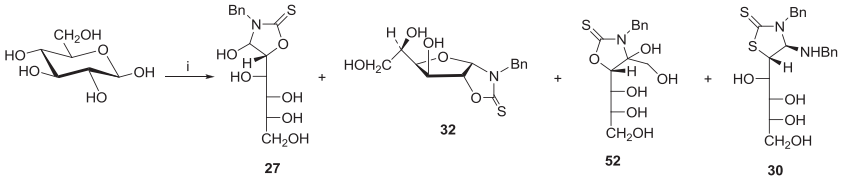


The above considerations clearly indicate that the chiral carbons in **32**, and hence in **27**, possess the same configuration as the starting D-glucose. This fact equally suggests that it is unnecessary to invoke a previous isomerization of D-glucose into D-fructose to explain the experimental results. This behavior is also reminiscent of data reported several decades ago by the teams of Hodge[44] and Kenne[49] for 2-*O*-phenylcarbamoyl-D-glucopyranose (**49**), generated by hydrolysis of **47** or **49**, which cyclizes under basic conditions to **50**. The latter can further be converted into a furanoid bicycle (**51**, Scheme 10), in following a similar trend to the reaction of D-glucosamine with BITC (Scheme 8).

The absolute stereochemistry of **29** has not yet been determined, this substance being reported as 3-benzyl-4-hydroxy-4-hydroxymethyl-5-(D-*erythro*-1,2,3-trihydroxypropyl)-1,3-oxazolidin-2-thione (surprisingly depicted as the *threo* isomer). It seems plausible to anticipate the *erythro* configuration for such a substance (**52**) because C-3, C-4, and C-5 carbon atoms possess the same configuration as the parent D-glucose. The absolute stereochemistry of **30** (D-*manno* configured) however could be determined by X-ray diffraction.[14] Scheme 11 should therefore reflect the actual situation found in the reaction of D-glucose with BITC.



Scheme 10. i, HCl 0.1 M; ii, H_2SO_4 1 M; iii, pH>7; iv, pH<7.



Scheme 11. i, BnNCS, NaOH, pH ~11, Δ (95 °C).

The formation of the 1,3-thiazolidin-2-thione derivative (**30**), according to Kruse and associates, was probably initiated by the reaction of benzylamine and hydrogen sulfide (liberated from the hydrolysis of BITC) with D-glucose giving transitional 2-sulfanyl-*N*-benzyl glucosylamine and, subsequently, a further amount of BITC was added to the thiol group formed and the reaction was completed intramolecularly.[14] However, this rationale does not account for the configurational inversion at C-2 that converts the D-*gluco* configuration into D-*manno*.

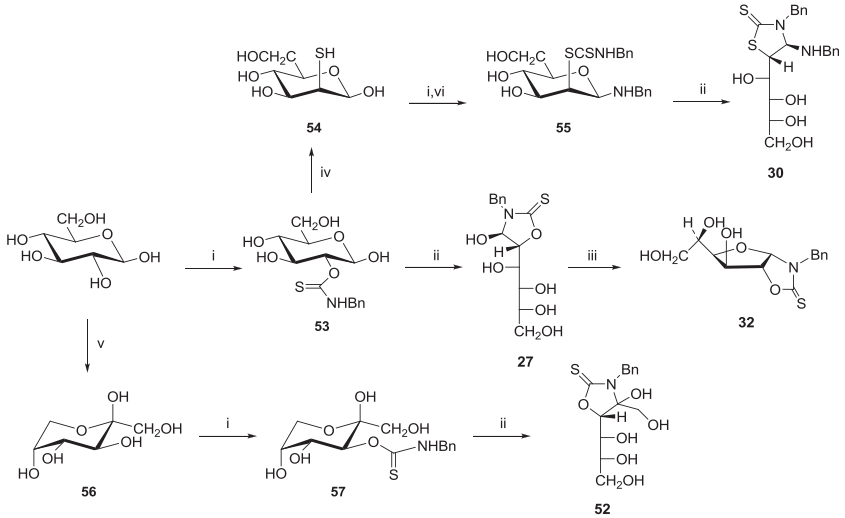
It is obvious that formation of **52** takes place after isomerization of D-glucose into D-fructose.[47,48] The generation of compounds **27**, **30**, **32**, and **52** can easily be rationalized by invoking the same benzylthiocarbamate intermediate (i.e., **53** or **57**), which may either cyclize into **27** or **52**, respectively, or undergo a S_N2 reaction that produces most likely 2-deoxy-2-sulfanyl-D-mannose (**54**), which further reacts with BITC giving rise to a benzyldithiocarbamate (**55**). Cyclization of the latter to yield **30** should largely be favored and proceed via a transient imine generated by reaction with benzylamine (Scheme 12).

Consumption of D-glucosamine and glucosinolate-rich vegetables may thus destroy the isothiocyanates resulting from glucosinolate hydrolysis, thereby reducing the beneficial effects attributed to such heterocumulenes.

4. Experimental

4.1. General methods

Melting points were determined on Gallenkamp and Electrothermal apparatus and are uncorrected. Optical rotations were measured at 22±5 °C on a Perkin–Elmer 241 polarimeter. IR spectra were recorded in the range 4000–600 cm^{-1} on FT-IR THERMO spectrophotometer. Solid samples were recorded on KBr (Merck) pellets. ^1H and ^{13}C NMR spectra were recorded on a Bruker 500 or with a Bruker 400 AC/PC instrument at 400 and 100 MHz, respectively, or with a Bruker AC 200-E instrument at 200 and 50.3 MHz, respectively, in different solvent systems. Assignments were confirmed by homo- and hetero-nuclear double-resonance

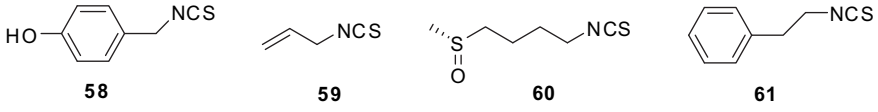


Scheme 12. i, BnNCS, NaOH, pH ~11, Δ(95 °C); ii, pH>7; iii, pH<7; iv, SH^-; v, OH^-; vi, $BnNH_2$.

3. Conclusions

The results reported in this paper provide a complete dissection on the reaction of BITC (a product of enzymatic hydrolysis of glucotropaeolin and inhibitor of carcinogenesis) with D-glucosamine (a common anti-inflammatory agent), and represents a plausible in vitro mimicry of this transformation under physiological conditions. All products have been thoroughly characterized and their formation can be explained through a mechanism that involves an initial thiourea as key step, which undergoes subsequent cyclization. This mechanistic proposal accounts for all the experimental facts and casts doubt on previous conjectures.

These results prove that ITCs, which may act as selective inhibitors of carcinogenesis, generated from other glucosinolates, such as 4-hydroxybenzyl isothiocyanate (**58**, from sinalbin[50]), allyl isothiocyanate (**59**, from sinigrin[51]), sulforaphane or 4-(*R*)-methylsulfinylbutyl isothiocyanate (**60**, from glucoraphanin[6b,52]) or 2-phenylethyl isothiocyanate (**61**, from gluconasturtiin[53]), among others, would indeed give rise to similar metabolites to those of BITC by reaction with D-glucosamine.



and DEPT or HMQC. TMS was used as the internal reference (δ=0.00 ppm) for $CDCl_3$ solutions only and all *J* values are given in hertz. Microanalyses were determined on a LECO 932 analyser at the Universidad de Extremadura (Spain). High resolution mass spectra (chemical ionization) were recorded on an Autospec-Q spectrometer by the Servicio de Espectrometría de Masas de la Universidad de Sevilla (Spain).

*4.1.1. (4R,5R)-1-Benzyl-5-hydroxy-4-(D-arabino-tetritol-1-yl)imidazolidin-2-thione (**3**) and 1-benzyl-(1,2-dideoxy-α-D-glucofurano)[2,1-d]imidazolidin-2-thione (**4**).* To a solution of 2-amino-2-deoxy-α-D-glucopyranose hydrochloride (**1**) (3.61 g, 16.8 mmol) in water (20 mL) were added $NaHCO_3$ (1.54 g, 18.5 mmol) and benzyl isothiocyanate (2.2 mL, 16.8 mmol) under stirring. The mixture was diluted with ethanol (30 mL) to obtain a homogenous solution that was then heated at 75 °C (external bath) for 2 h. When the mixture was left to room temperature *N*,*N*′-dibenzylthiourea (**2**) crystallized spontaneously as a white solid (0.80 g, 37%). Subsequent column chromatography of mother liquors (CH_2Cl_2–MeOH, 3:1) afforded **3** (1.48 g, 27%) and **4** (1.82 g, 35%).

Compound **3** have R_f 0.47 (CH_2Cl_2–MeOH 4:1), mp 83–84 °C (decomp.); $[\alpha]_D$ +8.0 (*c* 0.5, pyridine); ν_{max} 3500–3000 (OH, NH), 1638 (H_2O crystallization),[20] 1077 (C–O), 1490, and 780 cm^{-1} (aromatics); ^1H NMR (400 MHz, DMSO-d_6) δ 8.03 (s, 1H, NH), 7.36–7.24 (m, 5H, Ar), 6.65 (d, $J_{5,OH}$=8.0 Hz, 1H, C5–OH), 5.15 (d, *J*=15.6, 1H, *C*H_2–Ph), 4.93 (dd, $J_{4,5}$=3.2, $J_{5,OH}$=7.6 Hz, 1H, H-5), 4.68 (d, $J_{1',OH}$=6.4 Hz, 1H, C1′–OH), 4.54 (d, $J_{2',OH}$=8.0 Hz, 1H, C2′–OH), 4.52 (d, $J_{3',OH}$=7.6 Hz, 1H, C3′–OH), 4.40 (t, $J_{4',OH}$=$J_{4'',OH}$=5.6 Hz, 1H, C4′–OH), 4.35 (d, *J*=15.6 Hz, 1H, *C*H_2–Ph), 3.70–3.20 (m, 5H, 4H, H-1′, H-2′, H-3′, H-4′, H-4″); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.0 (C=S); 137.4, 128.5 (2C); 127.5 (2C); 126.8 (Ar); 83.7 (C-5), 70.7 (C1′), 70.6 (C2′); 69.1 (C3′); 64.2 (C4); 62.8 (C4′); 44.5 (CH_2). Anal. Calcd for $C_{14}H_{20}N_2O_5S\cdot H_2O$: C, 48.54; H, 6.40; N, 8.09; S, 9.26. Found: C, 48.49; H, 6.40; N, 7.85; S, 8.42. HRMS: *m*/*z* found 328.1072. [M^+] required for $C_{14}H_{20}N_2O_5S$ 328.1093.

Compound **4** have R_f 0.62 (CH_2Cl_2–MeOH 4:1), mp 85–86 °C (decomp.); $[\alpha]_D$ +45.5 (*c* 0.5, pyridine); ν_{max} 3480–2980 (OH, NH); 1474 (NH); 1634 (H_2O crystallization),[20] 1015, 970 (C–O); 774 and 727 cm^{-1} (aromatics); ^1H NMR (400 MHz, DMSO-d_6) δ 8.90 (s, 1H, NH), 7.34–7.25 (m, 5H, Ar), 5.59 (d, $J_{1,2}$=6.6 Hz, 1H, H-1), 5.25 (d, $J_{3,OH}$=5.2, 1H, C3–OH), 4.87 (d, *J*=15.6 Hz, 1H, *C*H_2–Ph), 4.69 (d, $J_{5,OH}$=6.0 Hz, 1H, C5–OH), 4.52 (d, *J*=15.2 Hz, 1H, *C*H_2–Ph), 4.39 (t, *J*=5.6 Hz, 1H, C6–OH), 4.02 (d, $J_{1,2}$=6.6 Hz, 1H, H-2), 4.00 (d, $J_{3,4}$=2.8 Hz, 1H, H-3), 3.63 (m, 1H, H-5), 3.44 (m, 1H, H-6), 3.33 (m, 1H, H-4), 3.19 (m, 1H, H-6′); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.4 (C=S); 137.9, 128.7 (2C); 128.1 (2C); 127.5 (Ar); 92.3 (C-1), 80.3 (C-4), 74.5 (C3′); 68.7 (C5); 65.1 (C2); 64.2 (C-6); 47.5 (CH_2). Anal. Calcd for $C_{14}H_{18}N_2O_4S\cdot\frac{1}{2}H_2O$: C, 52.65; H, 6.00; N, 8.77; S, 10.04. Found: C, 52.56; H, 6.24; N, 8.51; S, 9.85. HRMS: *m*/*z* found 310.0976. [M^+] required for $C_{14}H_{18}N_2O_4S$ 310.0987.

*4.1.2. (4R,5S)-1-Benzyl-5-hydroxy-4-(D-arabino-tetritol-1-yl)imidazolidin-2-thione (**5**).* A solution of **3** (0.03 g) in DMSO-d_6 (0.5 mL) was monitored by ^1H NMR, which detected the partial conversion into (4*R*,5*S*)-1-benzyl-5-hydroxy-4-(D-*arabino*-tetritol-1-yl)imidazolidin-2-thione (**5**), being **3** the most stable isomer in the equilibrium (82%). ^1H NMR (400 MHz, DMSO-d_6) δ 8.01 (s, 1H, NH), 7.36–7.24 (m, 5H, Ar), 6.52 (d, $J_{5,OH}$=7.6 Hz, 1H, C5–OH), 5.26 (d, *J*=15.2, 1H, *C*H_2–Ph), 5.00 (t, $J_{4,5}$=$J_{5,OH}$=7.3 Hz, 1H, H-5), 4.69 (d, $J_{1',OH}$=6.8 Hz, 1H, C1′–OH), 4.65 (d, $J_{2',OH}$=6.40 Hz, 1H, C2′–OH), 4.53 (d, $J_{3',OH}$=7.60 Hz, 1H, C3′–OH), 4.40 (t, $J_{4',OH}$=$J_{4'',OH}$=5.6 Hz, 1H, C4′–OH), 4.19 (d, *J*=1 5.6 Hz, 1H, *C*H_2–Ph), 3.70–3.20 (m, 5H, 4H, H-1′, H-2′, H-3′, H-4′, H-4″); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.8 (C=S); 138.0, 128.0 (2C); 127.2 (2C); 126.4 (Ar); 80.0 (C-5), 70.6 (C1′), 70.5 (C2′); 67.2 (C3′); 63.2 (C4); 59.5 (C4′); 44.5 (CH_2).

*4.1.3. 1-Benzyl-4-(D-arabino-tetritol-1-yl)imidazolin-2-thione (**6**). Method a.* A solution of **3** (0.274 g, 0.83 mmol) in 30% aqueous acetic acid (8.5 mL) was heated at ~95 °C (external bath) for 30 min. The mixture was then evaporated to dryness and the residue was crystallized from 96% aqueous ethanol. The solid afforded was filtered and washed with cold ethanol (0.080 g, 31%). Compound **6** have mp 176–178 °C (decomp.)(lit.[31] mp 180–182 °C); $[\alpha]_D$ −17.6 (*c* 0.5, pyridine); ν_{max} 3500–3000 (OH, NH), 1636 (C=C), 1084–1032 (C–O), 758 cm^{-1} (aromatics); ^1H NMR (400 MHz, DMSO-d_6) δ 11.93 (s, 1H, NH), 7.35–7.28 (m, 5H, Ar), 6.84 (s, 1H, H-5), 5.15 (d, *J*=14.8 Hz, 1H, *C*H_2–Ph), 5.10 (d, *J*=14.8 Hz, 1H, *C*H_2–Ph), 4.97 (d, $J_{1',OH}$=7.20 Hz, 1H, C1′–OH), 4.64 (dd, $J_{1',2'}$=1.60 Hz, $J_{1',OH}$=7.20 Hz, 1H, H1′), 4.56 (s, 1H, C2′–OH), 4.54 (s, 1H, C3′–OH), 4.37 (t, $J_{4',OH}$=$J_{4'',OH}$=5.20 Hz, 1H, C4′–OH), 3.57–3.37 (m, 4H, H2′, H3′, H4′, H4″); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.7 (C=S); 138.4, 129.6 (2C), 129.1 (2C), 128.7 (Ar); 132.1 (C4), 115.9 (C5), 74.2 (C2′), 72.2 (C3′); 65.3 (C1′); 64.5 (C4′); 49.8 (CH_2). HRMS: *m*/*z* found 311.1055. $[M+H]^+$ required for $C_{14}H_{19}N_2O_4S$ 311.1066.

From preparative TLC of mother liquors of crystallization (chloroform–methanol, 7:1) compound **4** was isolated (0.093 g, 36%).

Method b. To a solution of **1** (2.89 g, 13.4 mmol) in water (17 mL) were added $NaHCO_3$ (1.23 g, 14.6 mmol) and benzyl isothiocyanate (1.78 mL, 13.4 mmol) under stirring. The mixture was diluted with ethanol (70 mL) to obtain a homogenous solution that was then heated at 80 °C (external bath) for 2 h. When the mixture was left to room temperature spontaneously crystallized **2** as a white solid (0.087 g, 2.7%). Mother liquors were evaporated to dryness and the residue obtained was treated with 30% aqueous acetic acid (118 mL) and heated at ~100 °C (external bath) for 30 min. The mixture was then washed with chloroform (3×25 mL) and the aqueous phase was collected and evaporated to dryness. The residue was crystallized with ethanol and a solid mixture of **4** and **6** was afforded. Recrystallization with 96% ethanol yielded compound **6** (0.27 g, 6.4%). Preparative TLC of mother liquors (chloroform–methanol, 7:1) afforded compound **4** (0.32 g, 7.6%).

Method c. A suspension of **9** (5.0 g, 13.9 mmol) and potassium thiocyanate (1.37 g, 13.9 mmol) in water (14 mL) was heated 3 h at 100 °C. After cooling, a mixture of **6** and potassium oxalate was separated (4.04 g). Recrystallization from 70% ethanol (15 mL) gave pure **6** (2.51 g, 51%).

*4.1.4. N-Benzyl-β-D-mannopyranosylamine (**8**).* This compound was synthesized following a minor modification of the published procedure.[30] A suspension of D-mannose (18.0 g, 0.10 mol) and benzylamine (10.9 mL, 0.10 mol) in absolute ethanol (50 mL) was refluxed for 5 min. After cooling, a glassy solid was formed. Solvent was removed in vacuo and the syrup obtained was dissolved in hot ethanol (50 mL). The solid separated was filtered and washed with cool ethanol and ether (22.3 g; 83%); mp 130 °C (lit.[30] 131–132 °C).

*4.1.5. 1-Benzylamino-1-deoxy-D-fructopyranose oxalate (**9**).* This compound (76%) was synthesized following the published procedure.[30] M.p 158 °C (lit.[30] 157–159 °C).

*4.1.6. 4-(1,2,3,4-Tetra-O-acetyl-D-arabino-tetritol-1-yl)-1-benzylimidazolin-2-thione (**11**).* To a solution of **6** (0.62 g, 2.0 mmol) in pyridine (8.0 mL), cooled at −20 °C, was added acetic anhydride (8.0 mL) and the reaction mixture was kept at that temperature for 12 h. Then it was poured into ice-water and the resulting solid was filtered and washed with cold water and identified as a mixture of **11** and **13** (0.73 g, 78%). Recrystallization from absolute ethanol gave pure **11**; mp 73–75 °C (decomp.); $[\alpha]_D$ −24.0 (*c* 0.5, chloroform); ν_{max} 3481, 3224 (NH); 1752, 1711 (C=O, ester); 1630 (C=C), 1453, 1372 (CH_3), 1217 (C–O–C, ester); 1066, 1040, 1026 (C–O), 3138, 1455, 731, and 637 cm^{-1} (aromatics); ^1H NMR (500 MHz, Cl_3CD) δ 11.22 (s, 1H, NH), 7.39–7.29 (m, 5H, Ar), 6.58 (s, 1H, H-5), 5.95 (d, $J_{1',2'}$=3.5 Hz, 1H, H-1′), 5.44 (dd, $J_{1',2'}$=3.5 Hz, $J_{2',3'}$=8.0 Hz, 1H, H-2′), 5.24 (d, *J*=14.5 Hz, 1H, CH_2–Ph), 5.19 (ddd, $J_{2',3'}$=8.0 Hz, $J_{3',4'}$=2.5 Hz, $J_{3',4''}$=5.0 Hz, 1H, H-3′), 5.12 (d, *J*=14.5 Hz, 1H, CH_2–Ph), 4.25 (dd, $J_{4',4''}$=12.5 Hz, $J_{3',4'}$=2.5 Hz, 1H, H-4′), 4.11 (dd, $J_{4',4''}$=12.5 Hz, $J_{3',4''}$=5.0 Hz, 1H, H-4″), 2.11 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.05 (s, 6H, OAc), ^{13}C NMR (125 MHz, Cl_3CD) δ 170.5 (2C), 169.8 (2C) (CH_3CO), 169.4 (C=S), 135.3(Ar), 129.0 (2C, Ar), 128.3 (3C, Ar), 124.1 (C-4), 116.3 (C-5), 70.3 (C2′), 68.5 (C3′), 64.4 (C1′), 61.6 (C4′), 50.6 (*C*H_2Ph), 20.7 (2× *C*H_3CO), 20.6 (2× *C*H_3CO). Anal. Calcd for $C_{22}H_{26}N_2O_8S$: C, 55.26; H, 5.48; N, 5.86; S, 6.70. Found: C, 55.02; H, 5.46; N, 5.86; S, 6.70. HRMS: *m*/*z* found 479.1472. $[M+H]^+$ required for $C_{22}H_{27}N_2O_8S$ 479.1488.

*4.1.7. 3,5,6-Tri-O-acetyl-1-benzyl-(1,2-dideoxy-α-D-glucofurano)[2,1-d]imidazolidin-2-thione (**12**).* To a solution of **4** (0.42 g, 1.3 mmol) in pyridine (5.0 mL), cooled at −20 °C, was added acetic anhydride (3.5 mL) and the reaction mixture was kept at that temperature for 12 h. Then it was poured into ice-water and the resulting white solid was filtered and washed with cold water and ethanol and identified as **12** (0.43 g, 75%); mp 53–54 °C (decomp.); $[\alpha]_D$ +52.0 (*c*

0.5, chloroform); ν_{max} 3383 (NH); 1749 (C=O, ester); 1230 (C–O–C, ester); 1045 (C–O) 1455, 723 and 700 cm^{-1} (aromatics); ^1H NMR (400 MHz, Cl_3CD) δ 7.38–7.28 (m, 5H, Ar), 6.50 (s, 1H, NH), 5.67 (d, $J_{1,2}$=6.40 Hz, 1H, H-1), 5.22 (d, J=14.8 Hz, 1H, CH_2–Ph), 5.20 (m, 1H, H-5), 5.19 (d, $J_{3,4}$=2.80 Hz, 1H, H-3), 4.42 (d, J=14.8 Hz, 1H, CH_2–Ph), 4.41 (dd, $J_{6,6'}$=12.0 Hz, $J_{5,6}$=2.4 Hz, 1H, H-6), 4.11 (d, J=6.4 Hz, 1H, H-2), 4.06 (dd, $J_{3,4}$=2.8 Hz, $J_{4,5}$=9.20 Hz, 1H, H-4), 3.97 (dd, $J_{5,6'}$=5.6 Hz, $J_{6,6'}$=12.40 Hz, 1H, H-6′), 2.06 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.99 (s, 3H, OAc), ^{13}C NMR (100 MHz, Cl_3CD) δ 183.1 (C=S); 170.7 (CH_3CO), 169.9 (CH_3CO), 169.6 (2C) (CH_3CO); 136.2 (C2), 128.8 (2C), 128,6 (2C); 128.1 (Ar); 92.2 (C1), 76.1 (C3), 75.5 (C4), 67.3 (C5); 63.0 (C2); 62.9 (C6); 47.9 (CH_2Ph), 20.9, 20.8, 28.7 (3× CH_3CO). Anal. Calcd for $C_{20}H_{24}N_2O_7S$: C, 55.04; H, 5.54; N, 6.42; S, 7.35. Found: C, 54.91; H, 5.47; N, 6.54; S, 7.09. HRMS: m/z found 437.1386. $[M+H]^+$ required for $C_{20}H_{25}N_2O_7S$ 437.1382.

*4.1.8. 1-Acetyl-5-(1,2,3,4-tetra-O-acetyl-D-arabino-tetritol-1-yl)-3-benzylimidazolin-2-thione (**13**).* A mixture of **6** (0.62 g, 2.0 mmol) in pyridine (8.0 mL) and acetic anhydride (8.0 mL) was heated 3 h at 80 °C and then was kept at room temperature for 12 h. Then it was poured into ice-water and the resulting solid was filtered and washed with cold water (0.84 g, 78%). The sensitivity of the title compound to hydrolysis, yielding **11**, hampered the preparation of an analytical sample. ^1H NMR (400 MHz, Cl_3CD) δ 7.39–7.26 (m, 5H, Ar), 6.47 (s, 1H, H-4), 6.43 (d, $J_{1',2'}$=1.6 Hz, 1H, H-1′), 5.74 (d, J=14.8 Hz, 1H, CH_2–Ph), 5.49 (dd, $J_{1',2'}$=1.6 Hz, $J_{2',3'}$=9.6 Hz, 1H, H-2′), 5.21 (ddd, $J_{2',3'}$=9.6 Hz, $J_{3',4'}$=2.4 Hz, $J_{3',4''}$=5.2 Hz, 1H, H-3′), 4.72 (d, J=14.8 Hz, 1H, CH_2–Ph), 4.22 (dd, $J_{4',4''}$=12.8 Hz, $J_{3',4'}$=2.4 Hz, 1H, H-4′), 4.14 (dd, $J_{4',4''}$=12.8 Hz, $J_{3',4''}$=5.2 Hz, 1H, H-4″), 3.14 (s, 3H, NAc), 2.10 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.66 (s, 3H, OAc); ^{13}C NMR (100 MHz, Cl_3CD) δ 173.7, 170.5, 170.1, 169.8 (4× CH_3CO), 167.2 (C=S), 135.8 (Ar), 129.8 (2C, Ar), 129.2, 128.7 (2C, Ar), 126.5 (C-5), 118.2 (C-4), 69.4 (C1′), 68.8 (C2′), 67.3 (C3′), 62.9 (C4′), 51.4 (CH_2Ph), 28.9 (CH_3CON), 21.5, 21.4, 21.3, 20.8 (4× CH_3CO).

Acknowledgements

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Supplementary data

Schemes S1–S4 and Figs. S1–S15. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.07.073.

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