Electronic Supplementary Information (ESI)

Synthesis of thiodisaccharides related to 4-thiolactose. Specific structural modifications increase the inhibitory activity against the *E. coli* β-galactosidase

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General Information

Column chromatography was carried out with silica gel 60 (230–400 mesh). Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ aluminum-supported plates (layer thickness 0.2 mm). The spots were visualized by exposure to UV light and by charring with sulfuric acid (5% v/v in EtOH, containing 0.5% *p*-anisaldehyde). Optical rotations were measured at 25 °C in a 1 dm cell, in the solvent indicated. Nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz (¹H) or 125.7 MHz (¹³C). Chemical shifts were calibrated to tetramethylsilane or to a residual solvent peak. Assignments of ¹H and ¹³C NMR spectra were assisted by 2D ¹H-COSY or NOESY, and 2D ¹H-¹³C HSQC or HMBC experiments. High-resolution mass spectra (HRMS) were obtained using the electrospray ionization (ESI) technique and Q-TOF detection.

Determination of the configuration of thiodisaccharides based on NMR spectral data

The structure of the 3-deoxy-4-thio-hexopyranoside moiety of thiodisaccharides was deduced on the basis of their ¹H-NMR spectra, as follows.

Thiodisaccharide 6: The ¹H-NMR spectrum of **6** showed the H-1 signal as a doublet at 4.93 ppm with a coupling constant value ($J_{1,2} = 3.7$ Hz), typical of an axial-equatorial arrangement. The coupling of H-2 with H-3ax ($J_{2,3ax} = 12.0$ Hz) was characteristic of *trans* diaxial protons, while that with H-3eq ($J_{2,3eq} = 3.6$ Hz) agreed with their axial-equatorial orientation. Hence, an *R* configuration was assigned to the new stereocenter at C-2. Similarly, the coupling constant values $J_{3eq,4}$ (2.8 Hz), $J_{3ax,4}$ (2.8 Hz) and $J_{4,5}$ (2.5 Hz) were consistent with the *R* configuration for C-4.

Thiodisaccharide 7: The ¹H NMR spectrum of **7** revealed large *J* values for the coupling of H-4 with H-3ax ($J_{3ax,4} = 12.0$ Hz) and H-5 ($J_{4,5} = 11.5$ Hz) in agreement with a *trans*-diaxial orientation for all these protons, while H-4 and H-3eq ($J_{3eq,4} = 5.6$ Hz) maintained an axial-equatorial disposition. Comparison of the ¹H-NMR spectra of **4** and **7** revealed an upfield shifting of the H-4 signal from 3.70 ppm in **4** to 3.32 ppm in **7**. This could be attributed to the change in orientation of H-4 from an equatorial (in **4**) to an axial (in **7**) arrangement, which generally implies signal protection in such six-membered rings.¹

Thiodisaccharide 10: The ¹H-NMR spectrum of this compound showed a broad singlet (4.97 ppm) for H-2 and the downfield vinyl signals for H-4 and H-5 at 6.22 and 6.99 ppm, respectively. The coupling constant analysis suggested the enone adopted an ^o*E* conformation, since the small coupling constant value for the vinyl H-5 and allylic H-6 protons ($J_{5,6} = 1.6$ Hz) suggested an almost perpendicular arrangement among them. Furthermore, H-4 exhibited long-range couplings with H-2 (${}^{4}J_{2,4} = 0.3$ Hz) and with H-6 (${}^{4}J_{4,6} = 2.5$ Hz). All these *J* values were in agreement with those predicted by the Garbisch equation for the ${}^{\circ}E$ conformation of **10**.²

Thiodisaccharides 11 and 12: The configuration at C-4 in **11** and **12** was determined according to relevant coupling constants values, which are summarized in Table S2. The *p*-nitrobenzyl derivative **11**, similar to analogue **4**, showed relatively small coupling constant values for H-4 with H-3 and H-5 and a long-range coupling between H-1 and H-3eq, confirming the *R* configuration for C-4. The isomer **12** had opposite configuration at C-4, according to the large *J* values of H-4 with H-3ax and H-5.

Compound	Coupling constant (Hz)				
Compound	J _{3eq,4}	J 3ax,4	J 4,5	${}^4J_{1,3eq}$	J _{1',2'}
11	2.4	4.9	2.4	0.9	10.0
12	5.4	12.0	10.7	-	9.8

Table S2: Coupling constant values for uloses 11 and 12

Effect of Concentration of Thioglycomimetics 1 and 18 on the enzymatic activity of the β -galactosidase from *E. coli*.



References

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E. W. Garbisch, *J. Am. Chem. Soc.*, 1964, **86**, 5561–5564.





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¹³C-NMR Spectrum of compound 5 (125.7 MHz, CDCl₃).





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¹³C-NMR Spectrum of compound 7 (125.7 MHz, CDCl₃)





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¹³C-NMR Spectrum of compound 13 (125.7 MHz, CDCl₃)

























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¹H-NMR Spectrum of compound **21** (500 MHz, CDCl₃).



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¹H-NMR Spectrum of compound **22** (500 MHz, CDCl₃).













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¹H-NMR Spectrum of compound 25 (500 MHz, CDCl₃).



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