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Impact of glycosylation on physico–chemical and biological properties of nitrification inhibitors

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ABSTRACT

The lipophilic 2-mercaptobenzothiazole (**MBT**), known for its nitrification inhibition properties, was derivatized thanks to direct glycosylation reactions. Similar transformations were also performed starting from 2-mercaptobenzimidazole (**MBI**), structurally close to **MBT**. The resulting *S*-linked mono- or disaccharides derived from D-glucose or L-arabinose, and cellobiose, gentiobiose or lactose, respectively, were subsequently studied as novel nitrification inhibitors without any further formulation or physical processes, except dilution in water. Along with ecotoxicity measurements, inhibition properties of the synthesized water soluble glycoconjugates were studied in a model reactor containing nitrification bacteria. The best results were obtained for the gentiobiosyl derivatives simply dissolved in water.

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1. Introduction

Ammonium, formed by decomposition of organic nitrogen in soil or added as fertilizer, is rapidly oxidized to nitrite and nitrate in the nitrification process carried out by microorganisms. These anions are mobile in soils and readily lost from plant rooting zones by leaching or runoff, thus resulting in potential pollution of ground and surface waters.[1,2] Moreover, nitrate can also undergo denitrification in the absence of oxygen by other microorganisms to gaseous nitrogen oxides (e.g., N_2O one of the greenhouse gases involved in the stratospheric ozone layer depletion) and N_2.[3–5] These losses account for inefficiency in the use of applied nitrogen for plants. Indeed often less than 30% of the applied nitrogen fertilizers are recovered in intensive agricultural systems.[6,7] This international concern has stimulated researches for developing new compounds that effectively inhibit nitrification in soils in conjunction with the use of nitrogen fertilizers (urea, ammonium or other nitrogen sources).

Most of the potent nitrification inhibitors in soils are substituted heterocyclic *N*-compounds (e.g., pyridine, pyrazole, triazole, benzotriazole, imidazole …),[1] for which little is known about the mode of action. An ideal nitrification inhibitor should meet the requirements of being (i) non-toxic to other organisms, animals and humans, (ii) efficient at low concentrations (iii) mobile and stable in nutrient or fertilizer formulation, (iv) persistent to stay in soils for an adequate period (slow-and controlled-released to enhance efficiency of nutrients applied), and finally (v) cheap.[8,9] Among them, the 2-mercaptobenzothiazole (**MBT**) is known as a nitrification inhibitor[10,11] produced and marketed primarily in Japan whereas the analogous 2-mercaptobenzimidazole (**MBI**), structurally close to **MBT**, is not registered.[12] However, benzimidazole has been tested for this use and among many others unsubstituted heterocyclic *N*-compounds has a lower effect on nitrification.[1,13] Many nitrification inhibitors are incorporated into solid *N*-containing fertilizing materials. In these cases, the dissolution and suspending process may be aided by mechanical mixing, addition of suspending agents or other means.

Nevertheless, it is well known that glycosylation is amongst the most common process involved in nature to increase diversification. As a result, glycosylation provides many biological

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functions for both macromolecules and small molecules. Some of the latter glycoconjugates present clinical applications as bactericides, fungicides or in the treatment of cancers.[14] In this context, we propose herein to evaluate the impact of glycosylation on the physico–chemical and biological properties of the nitrification inhibitor **MBT** and of its **MBI** counterpart. We expect that the designed conjugates can maintain the inhibition activity of the starting material and/or can be partly hydrolyzed to release the desired activity. Thus, we describe herein the synthesis of various mono- or diglycosylated derivatives thereof (Fig. 1), their physico–chemical properties, and their ability to inhibit nitrification without further formulation or processes, except dilution in water.

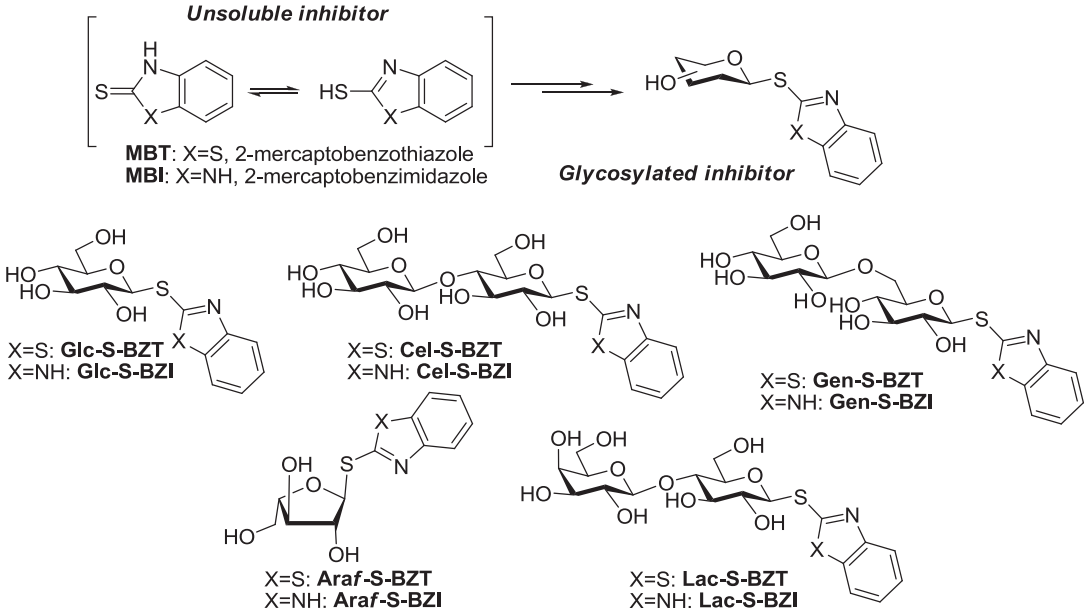


Fig. 1. Structures of new nitrification inhibitors, i.e., glycosylated **MBT** and **MBI**.

2. Results and discussion

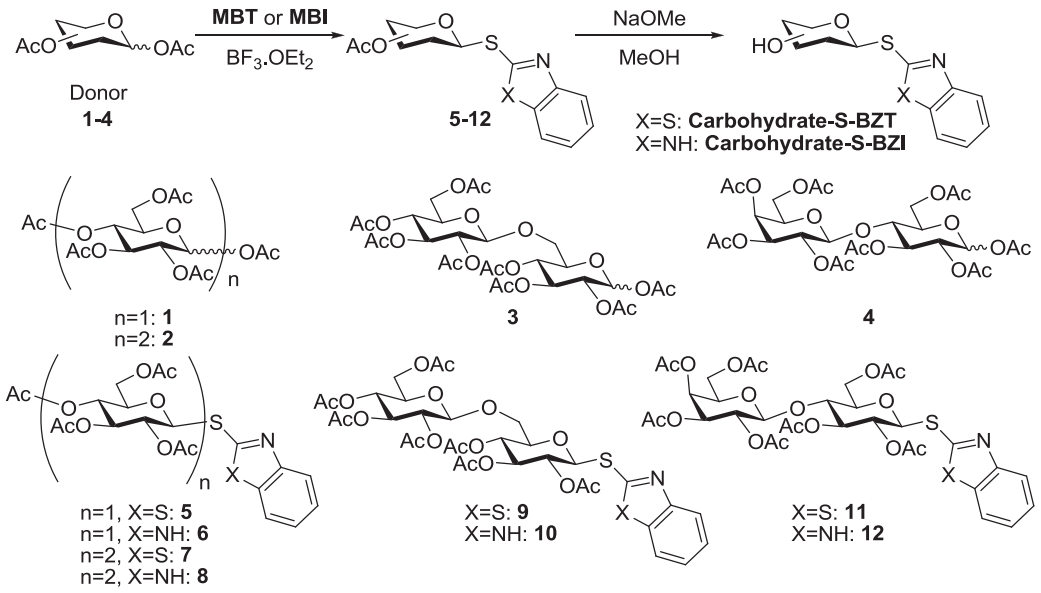
The targeted glycosylated 2-mercaptobenzothiazole **MBT** and 2-mercaptobenzimidazole **MBI** were prepared through a glycosylation-deprotection procedure starting from the readily available peracetylated mono- or disaccharides (Scheme 1). The coupling between the glycosyl donors **1**–**4** and the aromatic thiols were catalyzed by the boron trifluouride etherate complex and the deacylation step of the resulting thioimidates[15–17] was performed under Zemplen conditions (Table 1). Structural elucidation of the resulting products was deduced from ^{1}H, ^{13}C and 2D NMR analysis. Amongst selected data, the β-anomeric configuration was more particularly established on the basis of the coupling constant $J_{1,2}$ for the reducing residue. All observed coupling constants were found close to 10 Hz, that indeed reveals a 1,2-*trans* orientation between H-1 and H-2 in thioglycosides. The use of the arabinosyl counterparts[18] was also considered since arabinose is widely distributed in plants and microorganisms,[19,20] and because this carbohydrate is also found in a five-membered furanose form.

We further studied their solubility in water. While **MBT** (<0.03 g/L) and **MBI** (0.25 g/L) are weakly or non hydrosoluble at 25 °C, their glycosylation resulted in improved solubility in water [**Glc-S-BZT(BZI)**: 121(>200) g/L; **Cel-S-BZT(BZI)**: 19(>200) g/L; **Gen-S-BZT(BZI)** 21(>200) g/L; **Lac-S-BZT(BZI)**: 5(59) g/L]. More interestingly, this parameter showed to be strongly depending on the nature of the carbohydrate moiety. Considering the disaccharides, gentiobiose and cellobiose were much more interesting than lactose, whatever the aromatic aglycon. It was also observed that the monoglucosides **Glc-S-BZT(BZI)** are much more soluble in pure water than the disaccharides. This observation is nevertheless in good agreement with water solubility of the mono- or disaccharides.[21]

Subsequently, ecotoxicity measurements were performed. A first set of measurements was performed on a consortium of bacteria, more representative of the soil composition. The cocktail of bacteria used for this test came from activated mud collected from water treatment plants. The biological oxygen demand over 5 days (BOD_5) quantifies the amount of oxygen necessary for the biological degradation of organic residue in wastewater sample[22] and was calculated at a concentration of 100 μM for the selected **Gen-S-BZT** and **Gen-S-BZI**. Although the evaluation could not be performed for the insoluble **MBT**, it was previously shown that it can be removed by biological treatment when the effluent concentration is below 100–150 mg/L.[23] For the structurally close **MBI**, the measurement of chemical oxygen demand (COD) gave 29 mg/L O_2 and a −14 mg/L O_2 BOD_5 value. This revealed a decrease of the bacterial population over five days. On the other hand, the glycosylated compounds **Gen-S-BZT** (COD: 68 mg/L O_2, BOD_5: −4 mg/L O_2) and **Gen-S-BZI** (COD: 62 mg/L O_2, BOD_5: 6 mg/L O_2) demonstrated higher chemical and biochemical demands than that of the aglycons. This revealed a lesser impact on the bacterial population.

In order to strengthen the positive impact of the glycosylation of the aromatic compounds, a second evaluation relied on the impact of the active principles on bioluminescent *Vibrio fischeri*, a gram-negative and quorum-sensing bacterium sensitive to toxic compounds. The lowest EC_{50} were observed for **MBI** and **MBT** dissolved in water in the presence of non toxic polyethylene glycol (PEG). It was also shown that a significance improvement of the ecotoxicity (high EC_{50}) of glucosides, lactosides, cellobiosides, and more particularly gentiobiosides, whatever the nature of the aglycon (sevenfold better with **MBI** and more than 1500-fold better for the **MBT**). On the basis of the solubility tests and this first evaluation of the ecotoxicity, we further focused our attention on the gentiobosyl derivatives.

We thus evaluated the stability of **Gen-S-BZI** and **Gen-S-BZT** in solutions buffered at 5.5 or 8.0. These pHs were likely to mimic those found in soils. Under these hydrolytic conditions, both the *O*-



Scheme 1. Two-step preparation of glycosylated inhibitors.

Table 1
Results for targeted compounds synthesized according to Scheme 1

Entry	Glycosylation				Deprotection step	
	Donor	Acceptor	Product	Yield (%)	Product	Yield (%)
1	1	**MBT**	5	85	**Glc-S-BZT**	100
2	1	**MBI**	6	89	**Glc-S-BZI**	100
3	2	**MBT**	7	68	**Cel-S-BZT**	100
4	2	**MBI**	8	76	**Cel-S-BZI**	100
5	3	**MBT**	9[a]	—	**Gen-S-BZT**	28[b]
6	3	**MBI**	10	68	**Gen-S-BZI**	100
7	4	**MBT**	11	49	**Lac-S-BZT**	80
8	4	**MBI**	12	61	**Lac-S-BZI**	100

[a] Not isolated.
[b] For two steps.

and the *S*-glycosidic bonds may be cleaved to yield glucose, **Glc-S-BZI(BZT)**, gentiobiose and **MBI** (**MBT**). The monitoring of this degradation by LC-MS showed that **Gen-S-BZI** and **Gen-S-BZT** were more sensitive to acidic media. However, only traces of **MBT**/**MBI** could be detected by mass spectrometry under chemical hydrolysis conditions until 50 days, thus highlighting the great stability of our compounds in aqueous solutions.

Considering both the stability and the ecocompatibility of our glycoconjugates, we further studied their behaviour in the presence of bacteria able to consume ammonium ions and to produce nitrite species (*Nitrosomonas*) and nitrate anions (*Nitrobacter*). Some amounts of Maerl (1 g for 30 mL), a natural substance containing mineral elements, were added to the media in order to avoid pH variations. The activity of the inhibitors was compared to that of the pure aromatic compounds **MBT** and **MBI**, each at a concentration of 100 μM. Since the latter are not soluble in water, an additive was required. While ethanol enhance bacteria's growth even in very small quantities, the addition of polyethylene glycol (PEG) in moderation has proven particularly efficient in dissolving products without inducing adverse effects on the bacterial population. Subsequently, a first set of experiments was achieved using glycosylated derivatives and a consortium of bacteria stabilized after one month. The results were compared to a control experiment that contains neither heterocycles nor glycoconjugates. Surprisingly, no consumption of ammonium was observed under such conditions. When using **Gen-S-BZI(BZT)**, and after two days, LC/MS analysis failed to establish the presence of the starting glycosylated inhibitors in the medium, but the presence of **Glc-S-BZI(BZT)** was observed, even in very low concentration (1.3 μM for **Glc-S-BZI(BZT)**, versus 100 μM for the starting **Gen-S-BZI(BZT)**). We thus hypothesized that heterotrophic bacteria still present in the reactor were responsible for the observed fast degradation of the glycosides, since these bacteria are able to digest carbohydrates. Further digestion of **Gen-S-BZI(BZT)** thus liberated MBI(MBT) with their activity against the nitrification population. This experiment also showed, thanks to the presence of heterotrophic bacteria, the pro-inhibitor behaviour of the glycosylated compounds initially expected.

A second set of experiments was subsequently performed by stabilizing the bacterial population after six months in the dark, in order to strictly select autotrophic bacteria and to also avoid photosynthesis. Results in Fig. 2 showed that all ammonium ions were consumed after only two days without any inhibitor. This consumption has been significantly slow in the presence of **MBI** or **MBT** formulated in PEG solution, or with aqueous solutions of glycosylated inhibitors. While the lactosyl thioimidate **Lac-S-BZT** was less efficient, arabinosyl, glucosyl, cellobiosyl, and gentiobiosyl derivatives demonstrated interesting effects since more than 15 days were required when reactions were performed in their presence. Moreover, until two days, no significant difference between both active ingredients was noted. However, a significant difference in efficacy was observed beyond this time and it was interesting to note that **MBI**-conjugates provided better inhibition of *Nitrosomonas*. On another hand, an accumulation of nitrite anions was observed when the active part was **MBT**. This accumulation reached a peak at 8–10 days before decreasing when the ammonium concentration became too low. In this case, the inhibition of *Nitrobacter* was more important than that of *Nitrosomonas*. When the active part is **MBI**, there was no accumulation of nitrites. This was also related to the best inhibition of *Nitrosomonas* by **MBI** derivatives. As a conclusion, **MBI** and its conjugates appeared to be more suitable for agricultural applications than **MBT** and glycosylated-**MBT**.

3. Conclusion

The known nitrification inhibitor 2-mercaptobenzothiazole (**MBT**), and its structurally related analogue 2-mercaptobenz

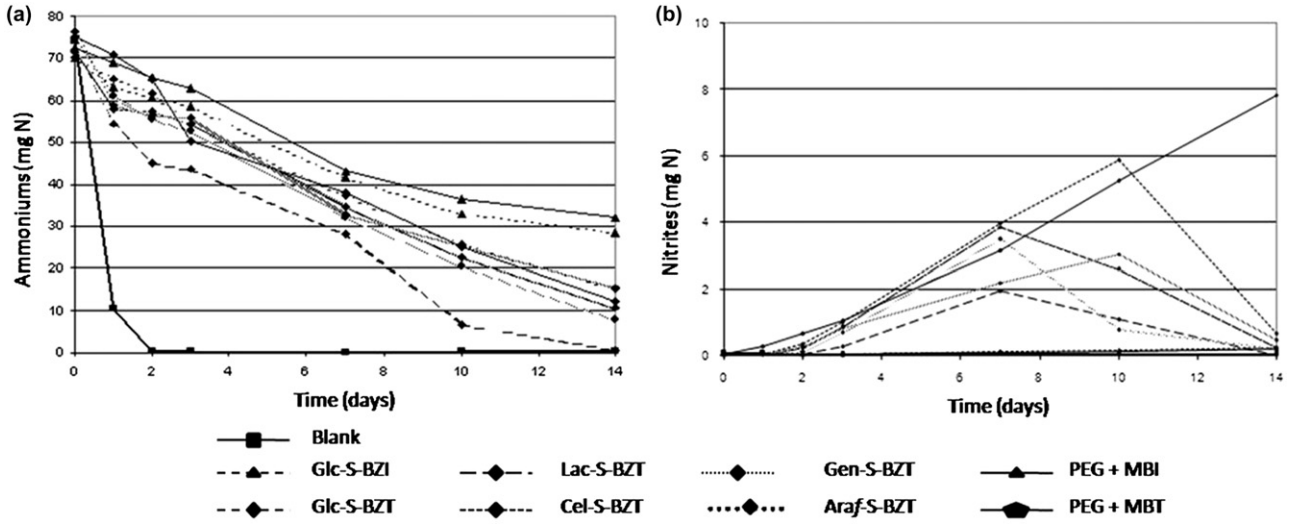


Fig. 2. Effects of inhibitors (100 μM) on (a) ammonium, and (b) nitrite concentrations.

imidazole (**MBI**), were easily glycosylated on a multi-gram scale. As expected, the introduction of one or two carbohydrate residues resulted in significant increase of solubility in water. Moreover, the gentiobiosyl conjugates showed good stability against chemical hydrolysis over a large period of 50 days and presented higher EC_{50} than the other derivatives, thus suggesting an improved ecotoxicity. As a result, the glycosylation seem not only to increase hydrosolubility but also to induce a better environmental tolerance compared to the native aromatic substrates, so that simplified formulations of nitrification inhibitors with low impact on the environment can be expected, even without any additives. This should simplify the agrochemical formulations to only bioactive compounds and so move towards more sustainable developments concepts. Moreover, glycoconjugates of the known inhibitor **MBT** and its structurally analogue **MBI** have shown similar nitrification inhibiting properties as the native compounds. In the presence of heterotrophic bacteria, which require organic compounds for their growth, **Gen-S-BZI(BZT)** were degraded, expectedly starting from the *O*-glycosidic linkage and followed by the *S*-glycosidic bond. This highlighted the pro-drug potency of the designed bioconjugates. Since glycosylation seem to provide various benefits for both physico–chemical properties and ecotoxicity, experiments in soils, which are much more complex media, will be performed in due course.

4. Experimental section

4.1. General

Thin layer chromatography (TLC) analyses were conducted on E. Merck 60 F_{254} Silica Gel non-activated plates. Anhydrous solvents were prepared and purified according to standard techniques just before use. Aromatic thiones, peracetylated mono- and disaccharides and other reagents were purchased from commercial suppliers and used as received. Compounds were visualized by UV (254 nm) and a 5% solution of orcinol in 5% solution of H_2SO_4 in EtOH, followed by heating. For column chromatography, Geduran Si 60 (40–63 μm) Silica Gel was used. Optical rotations were measured on a Perkin–Elmer 341 polarimeter. ^1H, ^{13}C, COSY, HSQC and TOCSY1d NMR spectra were recorded with a Brüker ARX 400 spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts are given in δ units (ppm). Coupling constants *J* were calculated in Hertz (Hz). Abbreviations were used to precise signal multiplicity: s (singlet), d (doublet), t (triplet), m (multiplet), dd (double doublet)… The high resolved mass spectra (HRMS) were measured at the 'Centre Regional de Mesures Physiques de l'Ouest (CRMPO, Université de Rennes 1)' with a MS/MS ZabSpec TOF Micromass using *m*-nitrobenzylic alcohol as a matrix and accelerated caesium ions for ionization. Microanalyses were also performed by the CRMPO.

4.2. General procedure for glycosylation reaction

To a solution of per-*O*-acetylated donor (1 equiv) in anhydrous dichloromethane were successively added the acceptor (**MBT** or **MBI**, 3 equiv) and $BF_3.OEt_2$ (9 equiv). The mixture was stirred at room temperature for 24 h. Then, the remaining acceptor was filtered off. The resulting filtrate was washed successively with a saturated solution of aqueous $NaHCO_3$ and water. The aqueous layers thus obtained were extracted with dichloromethane, and the combined organic layers finally dried over $MgSO_4$ and concentrated under reduced pressure. Flash chromatography on silica gel afforded the desired glycosyl thioimidate.

*4.2.1. Data for **5**.* Compound **5** was synthesized according to the general procedure starting from donor **1** (5.00 g, 12.8 mmol), 2-mercaptobenzothiazole (6.42 g, 38.4 mmol) and $BF_3.OEt_2$ (14.6 mL, 115.2 mmol). Column chromatography (9:1 Toluene/AcOEt) gave **5** (5.42 g, 10.9 mmol) as a white solid in 85% yield. TLC (4:1 toluene/AcOEt): R_f 0.4; $[\alpha]_D^{20}$+6.4 (*c* 1.25, CH_2Cl_2); mp 127–128 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (ddd, 1H, *J*=8.2 Hz, *J*=1.2 Hz, *J*=0.6 Hz, H_{arom}), 7.79 (ddd, 1H, *J*=8.0 Hz, *J*=1.2 Hz, *J*=0.6 Hz, H_{arom}), 7.46 (ddd, 1H, *J*=8.2 Hz, *J*=7.2 Hz, *J*=1.2 Hz, H_{arom}), 7.36 (ddd, *J*=8.0 Hz, *J*=7.2 Hz, *J*=1.2 Hz, H_{arom}), 5.58 (d, 1H, $J_{1,2}$=10.3 Hz, H-1), 5.35 (t, 1H, $J_{2,3}$=$J_{3,4}$=9.3 Hz, H-3), 5.23 (dd, 1H, H-2), 5.18 (dd, 1H, $J_{4,5}$=10.0 Hz, H-4), 4.31 (dd, 1H, $J_{6a,6b}$=12.5 Hz, $J_{5,6a}$=4.9 Hz, H-6a), 4.18 (dd, 1H, $J_{5,6b}$=2.3 Hz, H-6b), 3.93 (ddd, 1H, H-5), 2.05, 2.05, 2.04, 2.02 (4×s, 12H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 170.0, 169.4, 169.3 (CO), 161.8, 152.7, 135.8, 126.4, 125.0, 122.3, 121.1 (C_{arom}), 84.0 (C-1), 76.2 (C-5), 73.7 (C-3), 69.6 (C-2), 68.0 (C-4), 61.8 (C-6), 20.7, 20.6, 20.5 (CH_3); Calcd for $C_{21}H_{23}NO_9S_2$: C 50.70, H 4.66, N 2.82, S 12.89; Found C 50.53, H 4.67, N 2.80, S 12.91.

*4.2.2. Data for **6**.* Compound **6** was prepared as described starting from donor **1** (5.00 g, 12.8 mmol), 2-mercaptobenzimidazole

(5.81 g, 38.4 mmol) and $BF_3.OEt_2$ (14.6 mL, 115.2 mmol). Column chromatography (7:3 Toluene/AcOEt) afforded **6** (5.47 g, 11.4 mmol) as a white solid in 89% yield. TLC (4:1 light petroleum/AcOEt): R_f 0.5; $[\alpha]_D^{20}$ −22.1 (*c* 0.86, CH_2Cl_2); mp 138–139 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 2H, H_{arom}), 7.29–7.24 (m, 2H, H_{arom}), 5.27 (ddd, 1H, $J_{3,4}$=9.5 Hz, $J_{2,3}$=7.3 Hz, $J_{1,3}$=2.0 Hz, H-3), 5.12–5.06 (m, 3H, H-1, H-2, H-4), 4.38 (dd, 1H, $J_{6a,6b}$=12.5 Hz, $J_{5,6b}$=2.3 Hz, H-6b), 4.19 (dd, 1H, $J_{5,6a}$=4.7 Hz, H-6a), 3.79 (ddd, 1H, $J_{4,5}$=10.2 Hz, H-5), 2.11, 2.08, 2.05, 2.00 (4×s, 12H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 170.0, 169.5, 169.3 (CO), 143.5, 123.1 (C_{arom}), 83.4 (C-1), 76.4 (C-5), 73.4 (C-3), 70.0 (C-2), 67.9 (C-4), 61.6 (C-6), 20.9, 20.6, 20.5, 20.5 (CH_3); Calcd for $C_{21}H_{24}N_2O_9S$: C 52.49, H 5.03, N 5.83, S 6.67; Found C 52.46, H 5.04, N 5.84, S 6.54.

4.2.3. Data for ***7***. Disaccharide **7** was prepared as described starting from donor **2** (3.00 g, 4.42 mmol), 2-mercaptobenzothiazole (2.22 g, 13.2 mmol) and $BF_3.OEt_2$ (5.0 mL, 39.8 mmol). Column chromatography (3:2 Cyclohexane/AcOEt) gave **7** (2.37 g, 3.0 mmol) as a white solid in 68% yield. TLC (2:3 Cylohexane/AcOEt): R_f 0.6; $[\alpha]_D^{20}$ −19.2 (*c* 2.60, CH_2Cl_2); mp 205–206 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (ddd, 1H, *J*=8.3 Hz, *J*=1.1 Hz, *J*=0.6 Hz, H_{arom}), 7.78 (ddd, 1H, *J*=8.0 Hz, *J*=1.2 Hz, *J*=0.6 Hz, H_{arom}), 7.46 (ddd, 1H, *J*=8.3 Hz, *J*=7.2 Hz, *J*=1.2 Hz, H_{arom}), 7.35 (ddd, 1H, *J*=8.0 Hz, *J*=7.2 Hz, *J*=1.1 Hz, H_{arom}), 5.51 (d, 1H, $J_{1,2}$=10.3 Hz, H-1), 5.31 (t, 1H, $J_{2,3}$=$J_{3,4}$=9.1 Hz, H-3), 5.15 (t, 1H, $J_{3',4'}$=9.4 Hz, $J_{2',3'}$=9.2 Hz, H-3′), 5.14 (dd, 1H, H-2), 5.07 (t, 1H, $J_{4',5'}$=9.7 Hz, H-4′), 4.94 (dd, 1H, $J_{1',2'}$=7.9 Hz, H-2′), 4.55 (dd, 1H, $J_{6a,6b}$=12.1 Hz, $J_{5,6b}$=1.7 Hz, H-6b), 4.52 (d, 1H, H-1′), 4.38 (dd, 1H, $J_{6'a,6'b}$=12.5 Hz, $J_{5',6'a}$=4.4 Hz, H-6′a), 4.15 (dd, 1H, $J_{5,6a}$=4.9 Hz, H-6a), 4.05 (dd, $J_{5',6'b}$=2.3 Hz, H-6′b), 3.89–3.82 (m, 2H, H-4, H-5), 3.67 (ddd, 1H, $J_{4',5'}$=9.7 Hz, H-5′), 2.09, 2.06, 2.05, 2.04, 2.03, 2.01, 1.99 (7×s, 21H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 170.2, 169.6, 169.3, 169.1 (CO), 161.9, 152.7, 135.8, 126.4, 125.0, 122.4, 120.9 (C_{arom}), 100.8 (C-1′), 83.9 (C-1), 77.2, 76.2 (C-4, C-5), 73.3 (C-3), 72.9 (C-3′), 72.0 (C-5′), 71.6 (C-2′), 69.9 (C-2), 67.8 (C-4′), 61.9 (C-6), 61.6 (C-6′), 20.8, 20.7, 20.6, 20.5, 20.4 (CH_3); Calcd for $C_{33}H_{39}NO_{17}S_2$: C 50.44, H 5.00, N 1.78, S 8.16; Found C 50.31, H 5.05, N 1.73, S 8.13.

4.2.4. Data for ***8***. Disaccharide **8** was synthesized starting from donor **2** (3.00 g, 4.42 mmol), 2-mercaptobenzimidazole (1.99 g, 13.2 mmol) and $BF_3.OEt_2$ (5.0 mL, 39.8 mmol). Column chromatography (3:2 Cyclohexane/AcOEt) gave the desired compound **8** (2.58 g, 3.4 mmol, 76% yield) as a white solid. TLC (2:3 Cylohexane/AcOEt): R_f 0.4; $[\alpha]_D^{20}$ −30.5 (*c* 4.85, CH_2Cl_2); mp 144–146 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 2H, H_{arom}), 7.32–7.26 (m, 2H, H_{arom}), 5.22 (t, 1H, $J_{2,3}$=$J_{3,4}$=8.9 Hz, H-3), 5.16 (t, 1H, $J_{2',3'}$=9.4 Hz, $J_{3',4'}$=9.6 Hz, H-3′), 5.07 (t, 1H, $J_{4',5'}$=9.6 Hz, H-4′), 5.00 (dd, 1H, $J_{1,2}$=10.0 Hz, H-2), 4.95 (d, 1H, H-1), 4.93 (dd, 1H, $J_{1',2'}$=8.0 Hz, H-2′), 4.81 (dd, 1H, $J_{6a,6b}$=12.3 Hz, $J_{5,6b}$=2.2 Hz, H-6b), 4.57 (d, 1H, H-1′), 4.37 (dd, 1H, $J_{6'a,6'b}$=12.5 Hz, $J_{5',6'a}$=4.3 Hz, H-6′a), 4.13 (dd, 1H, $J_{5,6a}$=4.1 Hz, H-6a), 4.03 (dd, 1H, $J_{5',6'b}$=2.2 Hz, H-6′b), 3.78 (dd, 1H, $J_{4,5}$=9.3 Hz, H-4), 3.70–3.62 (m, 2H, H-5, H-5′), 2.16, 2.08, 2.06, 2.02, 2.01, 2.00, 1.98 (7×s, 21H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 170.5, 170.3, 169.8, 169.5, 169.2, 168.8 (CO), 143.2, 123.4 (C_{arom}), 100.6 (C-1), 82.9 (C-1′), 77.2 (C-5), 75.6, (C-4), 72.9 (C-3), 72.8 (C-3′), 72.1 (C-5′), 71.6 (C-2′), 70.3 (C-2′), 67.6 (C-4′), 61.4 (C-6′), 60.9 (C-6′$_6$), 21.1, 20.6, 20.5, 20.4 (CH_3); Calcd for $C_{33}H_{40}N_2O_{17}S$: C 51.56, H 5.24, N 3.64, S 4.17; Found C 51.26, H 5.27, N 3.30, S 3.80.

4.2.5. Data for ***9***. This intermediate was prepared starting from peracetylated gentiobiose **3** (2.00 g, 2.9 mmol), 2-mercaptobenzothiazole (1.48 g, 8.8 mmol) and $BF_3.OEt_2$ (3.4 mL, 26.5 mmol). The resulting product **9** was further engaged in the deprotection step after simple work-up but without chromatographic purification.

4.2.6. Data for ***10***. Gentiobioside **10** was obtained starting from donor **3** (2.00 g, 2.9 mmol), 2-mercaptobenzimidazole (1.33 g, 8.8 mmol) and $BF_3.OEt_2$ (3.4 mL, 26.5 mmol). Column chromatography (3:2 Cyclohexane/AcOEt) yielded the desired compound **10** (1.54 g, 2.0 mmol, 68%) as a white solid. TLC (2:3 Toluene/AcOEt): R_f 0.4; $[\alpha]_D^{20}$ −5.5 (*c* 4.00, CH_2Cl_2); mp 96–97 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.65 (s, 2H, H_{arom}), 7.34–7.28 (m, 2H, H_{arom}), 5.26 (t, 1H, $J_{2',3'}$=$J_{3',4'}$=9.6 Hz, H-3′), 5.25 (t, 1H, $J_{2,3}$=$J_{3,4}$=9.3 Hz, H-3), 5.09 (dd, 1H, $J_{1,2}$=10.1 Hz, $J_{2,3}$=9.3 Hz, H-2), 5.08 (dd, 1H, $J_{1',2'}$=8.0 Hz, H-2′), 5.02 (t, 1H, $J_{4,5}$=9.8 Hz, H-4), 4.98 (d, 1H, H-1), 5.04 (t, 1H, $J_{4',5'}$=9.9 Hz, H-4′), 4.61 (d, 1H, H-1′), 4.16 (dd, 1H, $J_{6'a,6'b}$=12.4 Hz, $J_{5',6'b}$=2.7 Hz, H-6′b), 4.11 (dd, 1H, $J_{5',6'a}$=4.6 Hz, H-6′a), 3.89 (dd, 1H, $J_{6a,6b}$=10.7 Hz, $J_{5,6b}$=2.0 Hz, H-6b), 3.79 (ddd, 1H, $J_{5,6a}$=6.5 Hz, H-5), 3.71 (dd, 1H, H-6a), 3.70 (ddd, 1H, H-5′), 2.10, 2.07, 2.05, 2.04, 2.03, 2.00, 1.98 (7×s, 21H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 170.3, 170.1, 170.0, 169.6, 169.4, 169.4 (CO), 144.2, 123.3 (C_{arom}), 100.5 (C-1′), 84.5 (C-1), 76.8 (C-5), 73.5, 72.3 (C-3, C-3′), 72.2 (C-5′), 70.7 (C-2′), 69.8 (C-2), 68.4, 68.2 (C-4, C-4′), 67.2 (C-6), 61.7 (C-6′), 20.8, 20.7, 20.6, 20.6, 20.6, 20.5 (CH_3); Calcd for $C_{33}H_{40}N_2O_{17}S$: C 51.56, H 5.24, N 3.64, S 4.17; Found C 51.40, H 5.29, N 3.91, S 3.98.

4.2.7. Data for ***11***. Lactosyl intermediate **11** was synthesized starting from donor **4** (1.00 g, 1.47 mmol), 2-mercaptobenzothiazole (0.74 g, 4.42 mmol) and $BF_3.OEt_2$ (1.7 mL, 13.2 mmol). Chromatographic purification (4:1 Toluene/AcOEt) yielded the desired compound **11** (0.57 g, 0.73 mmol, 49%) as a white solid. TLC (2:3 Toluene/AcOEt): R_f 0.7; $[\alpha]_D^{20}$ −9.0 (*c* 3.1, CH_2Cl_2); mp 167–169 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (ddd, 1H, *J*=8.2 Hz, *J*=1.2 Hz, *J*=0.6 Hz, H_{arom}), 7.78 (ddd, 1H, *J*=8.0 Hz, *J*=1.2 Hz, *J*=0.6 Hz, H_{arom}), 7.45 (ddd, 1H, *J*=8.2 Hz, *J*=7.3 Hz, *J*=1.2 Hz, H_{arom}), 7.35 (ddd, 1H, *J*=8.0 Hz, *J*=7.3 Hz, *J*=1.2 Hz, H_{arom}), 5.52 (d, 1H, $J_{1,2}$=10.3 Hz, H-1), 5.36 (dd, 1H, $J_{3',4'}$=3.5 Hz, $J_{4',5'}$=1.0 Hz, H-4′), 5.33 (t, 1H, $J_{2,3}$=$J_{3,4}$=8.6 Hz, H-3), 5.14 (dd, 1H, H-2), 5.12 (dd, 1H, $J_{2',3'}$=10.4 Hz, $J_{1',2'}$=7.9 Hz, H-2′), 4.96 (dd, 1H, H-3′), 4.52 (dd, 1H, $J_{6a,6b}$=12.0 Hz, $J_{5,6b}$=1.7 Hz, H-6b), 4.49 (d, 1H, H-1′), 4.15 (dd, 1H, $J_{5,6a}$=4.9 Hz, H-6a), 4.15–4.06 (m, 2H, H-6′a, H-6′b), 3.91–3.86 (m, 1H, H-5′), 3.89 (dd, 1H, $J_{4,5}$=9.9 Hz, H-4), 3.84 (ddd, 1H, H-5), 2.16, 2.06, 2.06, 2.05, 2.04, 2.03, 1.97 (7×s, 3H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 170.2, 170.1, 170.0, 169.6, 169.5, 169.0 (CO), 161.8, 152.7, 135.7, 126.3, 125.0, 122.3, 120.9 (C_{arom}), 101.0 (C-1′), 83.8 (C-1), 77.0 (C-5), 75.9 (C-4), 73.5 (C-3), 70.9 (C-3′), 70.7 (C-5′), 69.9 (C-2), 69.0 (C-2′), 66.6 (C-4′), 62.0 (C-6), 60.8 (C-6′), 20.8, 20.7, 20.6, 20.5 (CH_3); Calcd for $C_{33}H_{39}NO_{17}S_2$: C 50.44, H 5.00, N 1.78, S 8.16; Found C 50.62, H 5.11, N 1.88, S 3.84.

4.2.8. Data for ***12***. Lactosyl intermediate **12** was obtained from donor **4** (2.39 g, 3.5 mmol), 2-mercaptobenzimidazole (1.59 g, 10.6 mmol) and $BF_3.OEt_2$ (4.0 mL, 31.7 mmol). Chromatographic purification (7:3→1:1 Toluene/AcOEt) afforded compound **12** (1.64 g, 2.1 mmol) in 61% yield as a white solid. TLC (2:3 Toluene/AcOEt): R_f 0.5; $[\alpha]_D^{20}$ −25.1 (*c* 5.3, CH_2Cl_2); mp 106–108 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 2H, H_{arom}), 7.27–7.22 (m, 2H, H_{arom}), 5.34 (dd, 1H, $J_{3',4'}$=3.4 Hz, $J_{4',5'}$=1.1 Hz, H-4′), 5.24 (ddd, 1H, $J_{3,4}$=9.1 Hz, $J_{2,3}$=6.2 Hz, $J_{1,3}$=2.8 Hz, H-3), 5.11 (dd, 1H, $J_{2',3'}$=10.5 Hz, $J_{1',2'}$=7.9 Hz, H-2′), 5.03–4.96 (m, 2H, H-1, H-2), 4.98 (dd, 1H, H-3′), 4.77 (dd, 1H, $J_{6a,6b}$=12.2 Hz, $J_{5,6b}$=2.3 Hz, H-6b), 4.54 (d, 1H, H-1′), 4.13 (dd, 1H, $J_{5,6a}$=4.2 Hz, H-6a), 4.14–4.03 (m, 2H, H-6′a, H-6′b), 3.88 (ddd, 1H, $J_{5',6'a}$=7.4 Hz, $J_{5',6'b}$=6.4 Hz, H-5′), 3.80 (dd, 1H, $J_{4,5}$=9.9 Hz, $J_{3,4}$=9.1 Hz, H-4), 3.65 (ddd, 1H, H-5), 2.15, 2.14, 2.07, 2.04, 2.03, 2.02, 1.96 (7×s, 21H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 170.3, 170.1, 170.0, 169.8, 169.5, 168.9 (CO), 143.3, 123.0 (C_{arom}), 100.9 (C-1′), 83.0 (C-1), 77.2 (C-5), 75.5 (C-4), 73.3 (C-3),

70.8 (C-5′), 70.7 (C-3′), 70.4 (C-2), 69.1 (C-2′), 66.5 (C-4′), 61.1 (C-6), 60.7 (C-6′), 21.0, 20.7, 20.6, 20.5 (CH_3); Calcd for $C_{33}H_{40}N_2O_{17}S$: C 51.56, H 5.24, N 3.64, S 4.17; Found C 51.42, H 5.49, N 3.53, S 4.00.

4.3. General procedure for deacetylation

To a solution of per-*O*-acylated derivative in anhydrous methanol was added a 0.1 M solution of sodium methylate in methanol. The mixture was stirred at room temperature until no starting material was detected by TLC. Neutralisation was then performed by adding Amberlite IR-120 (H^+-form). The resin was finally filtered off and the solvent removed under reduced pressure to afford the desired product.

4.3.1. Data for ***Glc-S-BZT****.* Zemplen transesterification was performed from **5** (5.11 g, 10.3 mmol) using 0.1 M NaOMe/MeOH (10.3 mL). Work-up provided the desired product (3.38 g, 10.3 mmol, 100%) as a white powder. TLC (4:1 CH_2Cl_2/MeOH): R_f 0.7; $[\alpha]_D^{20}$ −72.5 (*c* 3.6, MeOH); mp 80–81 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.88 (d, 1H, J=8.0 Hz, H_{arom}), 7.83 (d, 1H, J=8.1 Hz, H_{arom}), 7.51 (t, 1H, J=7.7 Hz, H_{arom}), 7.42 (t, 1H, J=7.3 Hz, H_{arom}), 5.17 (d, 1H, $J_{1,2}$=9.1 Hz, H-1), 3.96 (dd, 1H, $J_{6a,6b}$=12.5 Hz, $J_{5,6b}$=2.0 Hz, H-6b), 3.79 (dd, 1H, $J_{5,6b}$=5.3 Hz, H-6a), 3.66–3.50 (m, 4H, H-2, H-3, H-4, H-5); ^{13}C NMR (100 MHz, D_2O) δ 164.7, 151.2, 134.9, 129.5, 125.1, 121.2, 120.9 (C_{arom}), 86.1 (C-1), 80.1 (C-5), 77.0 (C-3), 71.7 (C-2), 68.9 (C-4), 60.5 (C-6); ESI-HRMS for $[C_{13}H_{15}NO_5S_2+Na]^+$: calcd *m*/*z* 352.02894, found 352.0289.

4.3.2. Data for ***Glc-S-BZI****.* Deacylation of **6** (5.38 g, 11.2 mmol) using 0.1 M NaOMe/MeOH (11.2 mL) gave the target compound (3.44 g, 11.0 mmol, 100%) as a white solid. TLC (4:1 CH_2Cl_2/MeOH): R_f 0.5; $[\alpha]_D^{20}$ −53.6 (*c* 5.75, MeOH); mp 114–116 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.45–7.40 (m, 2H, H_{arom}), 7.21–7.16 (m, 2H, H_{arom}), 4.96 (d, 1H, $J_{1,2}$=9.9 Hz, H-1), 3.86 (dd, 1H, $J_{6a,6b}$=12.4 Hz, $J_{5,6b}$=2.2 Hz, H-6b), 3.69 (dd, 1H, $J_{6a,6b}$=12.4 Hz, $J_{5,6a}$=5.6 Hz, H-6a), 3.52 (t, 1H, $J_{2,3}$=$J_{3,4}$=8.9 Hz, H-3), 3.47 (ddd, 1H, $J_{4,5}$=9.8 Hz, H-5), 3.40 (dd, 1H, H-4), 3.35 (dd, 1H, H-2); ^{13}C NMR (100 MHz, D_2O) δ 145.6, 138.1, 123.0, 110.3 (C_{arom}), 85.5 (C-1), 80.1 (C-5), 76.9 (C-3), 71.9 (C-2), 69.0 (C-4), 60.5 (C-6); ESI-HRMS for $[C_{13}H_{16}N_2O_5S+Na]^+$: calcd *m*/*z* 335.06776, found 335.0673.

4.3.3. Data for ***Cel-S-BZT****.* Deprotection of **7** (2.36 g, 3.0 mmol) using 0.1 M NaOMe/MeOH (3.0 mL) gave **Cel-S-BZT** (1.46 g, 3.0 mmol, 100%) as a white solid. The latter was further recrystallized from MeOH/CH_2Cl_2 to afford the desired product (0.85 g, 1.7 mmol) in 58% yield. TLC (7:3 CH_2Cl_2/MeOH): R_f 0.5; $[\alpha]_D^{20}$ −121.6 (*c* 1.25, MeOH); mp 141–143 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.88 (ddd, 1H, J=8.3 Hz, J=1.1 Hz, J=0.6 Hz, H_{arom}), 7.84 (ddd, 1H, J=8.3 Hz, J=1.1 Hz, J=0.6 Hz, H_{arom}), 7.51 (ddd, 1H, J=8.3 Hz, J=7.3 Hz, J=1.1 Hz, H_{arom}), 7.42 (ddd, 1H, J=8.3 Hz, J=7.3 Hz, J=1.1 Hz, H_{arom}), 5.19 (d, 1H, $J_{1,2}$=9.9 Hz, H-1), 4.53 (d, 1H, $J_{1',2'}$=7.9 Hz, H-1′), 4.02 (d, 1H, $J_{6a,6b}$=11.7 Hz, H-6b), 3.92 (dd, 1H, $J_{6'a,6'b}$=12.4 Hz, $J_{5',6'b}$=2.2 Hz, H-6′b), 3.88 (dd, 1H, $J_{5,6a}$=2.9 Hz, H-6a), 3.78–3.69 (m, 3H, H-3, H-4, H-5), 3.73 (dd, 1H, $J_{5',6'a}$=5.6 Hz, H-6′a), 3.62 (dd, 1H, $J_{2,3}$=8.6 Hz, H-2), 3.52 (t, 1H, $J_{2',3'}$=$J_{3',4'}$=9.1 Hz, H-3′), 3.49 (ddd, 1H, $J_{4',5'}$=9.6 Hz, H-5′), 3.42 (dd, 1H, H-4′), 3.34 (dd, 1H, H-2′); ^{13}C NMR (100 MHz, D_2O) δ 164.7, 151.4, 135.2, 126.8, 125.4, 121.5, 121.1 (C_{arom}), 102.5 (C-1′), 86.0 (C-1), 79.0 (C-5), 77.8 (C-3), 76.0 (C-5′), 75.5 (C-3′), 75.4 (C-4), 73.1 (C-2′), 71.5 (C-2′), 69.4 (C-4′), 60.5 (C-6′), 59.8 (C-6); ESI-HRMS for $[C_{19}H_{25}NO_{10}S_2+Na]^+$: calcd *m*/*z* 514.08176, found 514.0814.

4.3.4. Data for ***Cel-S-BZI****.* Deprotection of **8** (2.46 g, 3.2 mmol) was performed with 0.1 M NaOMe/MeOH (3.2 mL) and afforded the cellobiosyl derivative **Cel-S-BZI** (1.49 g, 3.1 mmol, 100%) as a white solid. The latter was further recrystallized from MeOH/AcOEt to give the desired product (0.66, 1.4 mmol). TLC (3:1 CH_2Cl_2/MeOH): R_f 0.6; $[\alpha]_D^{20}$ −53.2 (*c* 2.50, MeOH); mp 159–160 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.63 (dd, 2H, J=6.1 Hz, J=3.2 Hz, H_{arom}), 7.38 (dd, 2H, J=6.1 Hz, J=3.2 Hz, H_{arom}), 5.12 (d, 1H, $J_{1,2}$=9.9 Hz, H-1), 4.50 (d, 1H, $J_{1',2'}$=7.9 Hz, H-1′), 3.97 (d, 1H, $J_{6a,6b}$=12.0 Hz, H-6b), 3.90 (dd, 1H, $J_{6'a,6'b}$=12.3 Hz, $J_{5',6'b}$=2.1 Hz, H-6′b), 3.84 (dd, 1H, $J_{5,6a}$=2.8 Hz, H-6a), 3.72 (dd, 1H, $J_{5',6'a}$=5.7 Hz, H-6′a), 3.71–3.68 (m, 3H, H-3, H-4, H-5), 3.50 (t, 1H, $J_{2',3'}$=$J_{3',4'}$=9.1 Hz, H-3′), 3.50–3.44 (m, 2H, H-5′, H-2), 3.41 (dd, 1H, $J_{4',5'}$=9.6 Hz, $J_{3',4'}$=9.1 Hz, H-4′), 3.31 (dd, 1H, H-2′); ^{13}C NMR (100 MHz, D_2O) δ 145.5, 138.1, 123.1, 114.3 (C_{arom}), 102.4 (C-1′), 85.4 (C-1), 79.0 (C-5), 77.9 (C-3), 75.9 (C-5′), 75.5 (C-3′), 75.4 (C-4), 73.0 (C-2′), 71.7 (C-2), 69.4 (C-4′), 60.5 (C-6′), 59.9 (C-6); ESI-HRMS for $[C_{19}H_{26}N_2O_{10}S+Na]^+$: calcd *m*/*z* 497.12059, found 497.1205.

4.3.5. Data for ***Gen-S-BZT****.* The crude product **9** was subjected to deprotection using 0.1 M NaOMe/MeOH (2.9 mL). The resulting material was purified by colomn chromatography on silica gel (7:3 CH_2Cl_2/MeOH) to give **Gen-S-BZT** as a white solid in a 28% yield (0.41 g, 0.83 mmol). TLC (7:3 CH_2Cl_2/MeOH): R_f 0.3; $[\alpha]_D^{20}$ −81.4 (*c* 2.85, MeOH); mp 128–131 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.89 (d, 1H, J=8.0 Hz, H_{arom}), 7.84 (d, 1H, J=8.2 Hz, H_{arom}), 7.51 (ddd, 1H, J=8.2, J=7.5, J=0.9 Hz, H_{arom}), 7.42 (ddd, 1H, J=8.0, J=7.4, J=0.9 Hz, H_{arom}), 5.23 (d, 1H, $J_{1,2}$=9.3 Hz, H-1), 4.42 (d, 1H, $J_{1',2'}$=7.6 Hz, H-1′), 4.19 (dd, 1H, $J_{6a,6b}$=11.9 Hz, $J_{5,6b}$=1.7 Hz, H-6b), 3.89 (dd, 1H, $J_{5,6a}$=5.4 Hz, H-6a), 3.82 (d, 1H, $J_{6'a,6'b}$=12.2 Hz, H-6′b), 3.82–3.75 (m, 1H, H-5), 3.62 (dd, 1H, $J_{5',6a'}$=3.3 Hz, H-6′a), 3.61–3.52 (m, 3H, H-2, H-3, H-4), 3.31–3.25 (m, 3H, H-3′, H-4′, H-5′), 3.25–3.18 (m, 1H, H-2′); ^{13}C NMR (100 MHz, D_2O) δ 164.5, 151.5, 135.2, 126.8, 125.4, 121.5, 121.2, (C_{arom}), 102.3 (C-1′), 86.1 (C-1), 79.4 (C-5), 76.9 (C-3), 75.8 (C-5′), 75.5 (C-3′), 73.0 (C-2′), 71.6 (C-2), 69.4 (C-4′), 68.8 (C-4), 68.0 (C-6), 60.6 (C-6′); ESI-HRMS for $[C_{19}H_{25}NO_{10}S_2+Na]^+$: calcd *m*/*z* 514.08176, found 514.0811.

4.3.6. Data for ***Gen-S-BZI****.* Deprotection of disaccharide **10** (1.47 g, 1.9 mmol) was performed with 0.1 M NaOMe/MeOH (1.9 mL) and afforded the desired derivative **Gen-S-BZI**, which was chromatographically purified (7:3 CH_2Cl_2/MeOH) and isolated as a white solid (0.90 g, 1.9 mmol, 100%). TLC (7:3 CH_2Cl_2/MeOH): R_f 0.3; $[\alpha]_D^{20}$ −45.8 (*c* 3.95, MeOH); mp 134–136 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.60 (dd, 2H, J=6.0 Hz, J=3.2 Hz, H_{arom}), 7.33 (dd, 2H, J=6.0 Hz, J=3.2 Hz, H_{arom}), 5.16 (d, 1H, $J_{1,2}$=9.9 Hz, H-1), 4.41 (d, 1H, $J_{1',2'}$=7.2 Hz, H-1′), 4.15 (dd, 1H, $J_{6a,6b}$=11.9 Hz, $J_{5,6b}$=1.5 Hz, H-6b), 3.90 (dd, 1H, $J_{5,6a}$=5.8 Hz, H-6a), 3.83 (d, 1H, $J_{6'a,6'b}$=12.1 Hz, H-6′b), 3.74 (ddd, 1H, $J_{4,5}$=9.1 Hz, H-5), 3.58 (t, 1H, $J_{2,3}$=$J_{3,4}$=9.1 Hz, H-3), 3.57 (dd, 1H, $J_{5',6a'}$=2.9 Hz, H-6′a), 3.50 (t, 1H, H-4), 3.44 (dd, 1H, H-2), 3.31–3.21 (m, 4H, H-2′, H-3′, H-4′, H-5′); ^{13}C NMR (100 MHz, D_2O) δ 145.8, 123.2 (C_{arom}), 102.4 (C-1′), 85.5 (C-1), 79.2 (C-5), 76.9 (C-3), 75.8, 75.5 (C-3′, C-5′), 73.0 (C-2′), 71.9 (C-2), 69.5 (C-4′), 69.0 (C-4), 68.5 (C-6), 60.6 (C-6′); ESI-HRMS for $[C_{19}H_{26}N_2O_{10}S+Na]^+$: calcd *m*/*z* 497.12059, found 497.1199.

4.3.7. Data for ***Lac-S-BZT****.* Product **11** (3.55 g, 4.2 mmol) was deacylated using 0.1 M NaOMe/MeOH (4.2 mL). The resulting material was purified by recrystallization from water to afford **Lac-S-BZT** as a white solid in an 80% yield (1.66 g, 3.4 mmol). TLC (7:3 CH_2Cl_2/MeOH): R_f 0.5; $[\alpha]_D^{20}$ −23.3 (*c* 3.0, DMSO); mp 186–187 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (ddd, 1H, J=7.9 Hz, J=1.2 Hz, J=0.5 Hz, H_{arom}), 7.88 (ddd, 1H, J=8.1 Hz, J=1.0 Hz, J=0.5 Hz, H_{arom}), 7.48 (ddd, 1H, J=8.1 Hz, J=7.2 Hz, J=1.2 Hz, H_{arom}), 7.39 (ddd, 1H, J=7.9 Hz, J=7.2 Hz, J=1.0 Hz, H_{arom}), 5.81 (d, 1H, J=6.3 Hz, OH-2), 5.19 (d, 1H, $J_{1,2}$=9.9 Hz, H-1), 5.11 (d, 1H, J=4.4 Hz, OH-2′), 4.88 (d, 1H, J=1.2 Hz, OH-4′), 4.80 (d, 1H, J=5.2 Hz, OH-3), 4.66 (d, 1H, J=5.14 Hz, OH-6), 4.65 (dd, 1H, J=5.4 Hz, J=1.1 Hz, OH-6′), 4.53 (d, 1H, J=4.6 Hz, OH-3′), 4.27 (d, 1H, $J_{1',2'}$=7.3 Hz, H-1′), 3.80 (ddd, 1H, $J_{6a,6b}$=12.0 Hz, J=5.5 Hz, J=2.1 Hz, H-6b), 3.71 (ddd, 1H, J=6.6 Hz, J=4.2 Hz, H-6a), 3.63 (dd, 1H, $J_{2',3'}$=4.3 Hz, $J_{3',4'}$=3.0 Hz,

H-3′), 3.58–3.44 (m, 6H, H-4, H-5, H-4′, H-5′, H-6′a, H-6′b), 3.39–3.29 (m, 3H, H-2, H-3, H-2′); ^{13}C NMR (100 MHz, D_2O) δ 164.5, 152.2, 135.0, 126.3, 124.5, 121.6, 121.3 (C_{arom}), 103.6 (C-1′), 85.9 (C-1), 79.6 (C-5), 79.2 (C-4′), 76.0 (C-3′), 75.5 (C-5′), 73.1 (C-4), 72.2 (C-2′), 70.5 (C-2), 68.1 (C-3), 60.3 (C-6), 60.0 (C-6′); ESI-HRMS for $[C_{19}H_{25}NO_{10}S_2+Na]^+$: calcd m/z 514.08176, found 514.0823.

4.3.8. Data for ***Lac-S-BZI***. Deacylation of **12** (1.64 g, 2.1 mmol) was performed with 0.1 M NaOMe/MeOH (2.1 mL) and afforded the lactosyl derivative **Lac-S-BZI** (1.49 g, 3.1 mmol, 100%) as a white solid. The latter was further recrystallized from MeOH/toluene afforded the desired derivative isolated as a white solid (0.45 g, 0.95 mmol, 44%). TLC (7:3 CH_2Cl_2/MeOH): R_f 0.4; $[\alpha]_D^{20}$ −48.5 (*c* 1.65, MeOH); mp 163–164 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.61 (dd, 2H, J=6.1 Hz, J=3.2 Hz, H_{arom}), 7.34 (dd, J=6.1 Hz, J=3.2 Hz, H_{arom}), 5.11 (d, 1H, $J_{1,2}$=9.9 Hz, H-1), 4.44 (d, 1H, $J_{1',2'}$=7.8 Hz, H-1′), 3.97 (dd, 1H, $J_{6a,6b}$=12.4 Hz, $J_{5,6b}$=1.4 Hz, H-6b), 3.92 (dd, 1H, $J_{3',4'}$=3.4 Hz, $J_{4',5'}$=0.7 Hz, H-4′), 3.83 (dd, 1H, $J_{5,6a}$=3.9 Hz, H-6a), 3.80–3.74 (m, 2H, H-6′a, H-6′b), 3.73–3.68 (m, 4H, H-3, H-4, H-5, H-5′), 3.66 (dd, 1H, $J_{2',3'}$=10.0 Hz, H-3′), 3.55 (dd, 1H, H-2′), 3.46 (ddd, 1H, $J_{2,3}$=6.5 Hz, $J_{2,4}$=2.3 Hz, H-2).; ^{13}C NMR (100 MHz, D_2O) δ 145.7, 123.3 (C_{arom}), 102.8 (C-1′), 85.4 (C-1), 79.0, 77.5, 75.6, 75.3 (C-3, C-4, C-5, C-5′), 72.5 (C-3′), 71.7 (C-2), 70.9 (C-2′), 68.5 (C-4′), 61.0 (C-6′), 59.9 (C-6); ESI-HRMS for $[C_{19}H_{26}N_2O_{10}S+Na]^+$: calcd m/z 497.12059, found 497.1200.

4.4. LC/MS analysis

Mass spectrometry analysis was carried out on a Quattro Premier (Micromass, Manchester, UK) equipped with an electrospray source (ESI Z-spray™) and a triple quadrupole. The nebulizer and desolvation gas was nitrogen generated from the nitrogen generator NM30LA (Peak scientific, Inchinnam, UK) and the collision gas was high grade argon (Air Liquide, Paris, France). The ESI source parameters were as follows: capillary voltage, 3 kV; source temperature, 120 °C; cone gas flow, 50 L/h, desolvation gas temperature, 350 °C; desolvation gas flow, 750 L/h; extraction voltage, 1 V; RF lens voltage, 0.3 V. Table 1 summarizes the method parameters for the molecules detection. The UPLC/MS/MS was controlled by the software Mass Lynx™ and data processing was performed by the program Target Lynx.

Analyte	Ionization	Mass transition	Cone voltage (V)	Collision energy (V)
Glucose	ESI−	179/89	20	5
Gentiobiose	ESI−	342/179	20	10
Glc-S-BZT	ESI+	330/168	17	13
Gen-S-BZT	ESI+	492/168	20	15
Glc-S-BZI	ESI+	313/151	30	20
Gen-S-BZI	ESI+	475/151	20	12

4.5. Ecotoxicity of (a) MBI-derivatives and (b) MBT-conjugates

Impact of the active principles was measured on bioluminescent *Vibrio fischeri*. The results obtained on a Microtox Acute apparatus are expressed in EC_{50}, the concentration that kills half of the bacterial population. The survival of the remaining bacteria was measured at 490 nm.

4.6. Cell culture

The required installation was developed from a biological floc (20 L) taken from the aeration tank of a wastewater purification (Cesson-Sévigné, Ille-et-Vilaine, France), which was first sifted throw a 0.5 mm sieve. The filtrate was decanted and the resulting supernatant was withdrawn. The concentrated solution thus obtained was put in an aerobic reactor, using a continuous aeration. Bacteria were fed continuously, and consumed each week a solution of 50 L of water containing 32 g of NH_4HCO_3, 30 g $NaHCO_3$, 4 g Na_2HPO_4 and a spatula tip of iron-II chloride. After one or six months, the consortium of bacteria can be used for inhibition tests.

4.7. Preparation of bacteria solution

A 500 mL of bacteria solution was taken from the reactor. After a first decantation, and removal of the supernantant, ultrapure water (200 mL) was added to the resulting mixture of bacteria. The suspension was then stirred until homogenization, decanted and the supernatant removed. This procedure was further repeated three times more. The resulting sample was subsequently used for the inhibition tests.

4.8. Inhibition

For each inhibitor, a 30 mL of the solution containing ammonium chloride (100 mg/L in NH_4^+), inhibitor (100 μM), and finally 1 mL of bacteria solution was prepared in a test tube containing 1 g of maerl (1 g). The blank solution consisted in the same mixture but without organic compounds. All mixtures were prepared in parallel. Test tubes were stirred at 400 rpm and room temperature. Measurements of pH, ammonium, nitrite, nitrate concentrations and cell viability were achieved as follows.

4.9. Cell viability

Determination of ammoniums, nitrite and nitrate concentrations: The desired concentrations were further determined starting from 1 mL of samples, taken under stirring, which were filtrated through filter paper. The resulting filtrate was diluted with ultrapure water (5–100). The concentration in ammonium cations was obtained photometrically, using the ammonium Test from Merck (Ref 1.14,752.0001). The concentration in nitrites was determined either photometrically at 537 nm using a diazotation reactive (sulfanilamide and *N*-(1-naphtyl)ethylene diamine solution in water), or by ion chromatography. Nitrates concentration was determined by ion chromatography.

4.10. Ion chromatographic analysis

The samples (250 μL) were injected into a DX 120 system (Dionex) equipped with an Ion Pac AS 18 column (4 x 250 mm, Ref 062885), a precolumn AG 18 (Ref 062887) and a suppressor ASRS 300 (50 mA, 4 mm, P/N 064554) and eluted at a flow rate of 1 mL/min with a gradient solution of KOH generated in situ by an EGC-KOH cartridge. The gradient was as follows: 10 mM KOH from 0 to 10 min, an increase from 10 mM to 45 mM KOH from 10 to 25 min, then 45 mM KOH until 35 min. The detection by conductivity happened after electrolytic suppression. The system was controlled by the software Chromeleon.

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