Tetrahedron 68 (2012) 7095–7102

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

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

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ARTICLE INFO

Article history:
Received 28 February 2012
Received in revised form 11 June 2012
Accepted 14 June 2012
Available online 21 June 2012

Keywords:
Glycosylation
Nitrification inhibitor
Ecotoxicity
2-Mercaptobenzothiazole
2-Mercaptobenzimidazole

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.

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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0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2012.06.060

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.

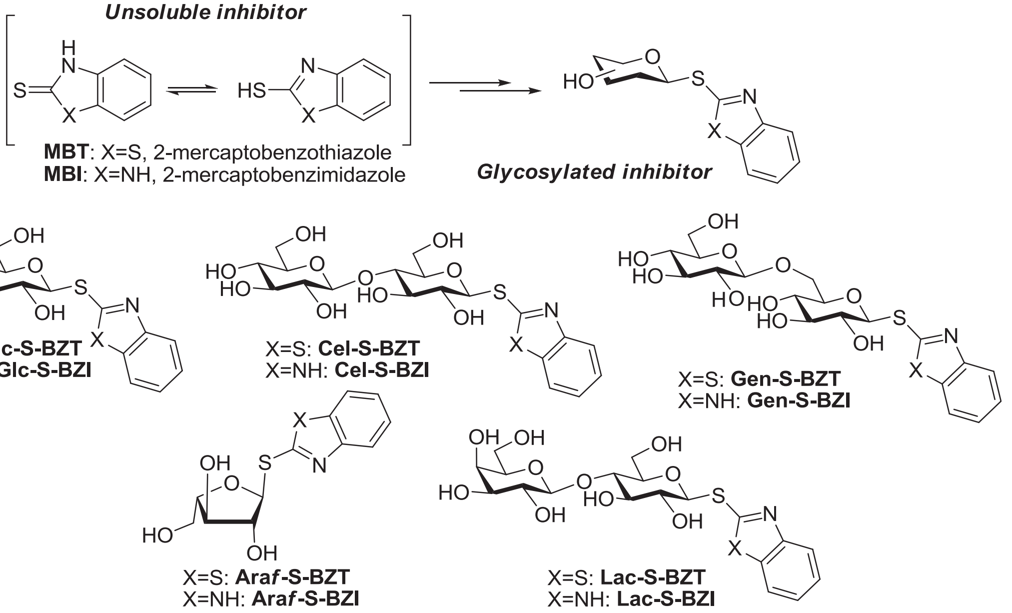

Fig. 1. Structures of new nitrification inhibitors, i.e., glycosylated **MBT** and **MBI**.

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*-