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# Cationic derivatives of the plant resistance inducer benzo[1,2,3]thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) as bifunctional ionic liquids

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ABSTRACT

We have synthesized, new salt derivatives of benzo[1,2,3]thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) containing BTH as the cation in combination with anions, which modify the physical and antibacterial properties of the resulting product while preserving the systemic acquired resistance (SAR) induction properties. The obtained compounds were tested toward induction of resistance in tobacco (*Nicotiana tabacum* var. *Xanthi*), which was later infected with OLV-1 and TMV viruses. Moreover *in vitro* tests on bacteria confirmed the preservation of antibacterial properties brought about by a docusate anion.



Crop protection in modern agricultural industry, despite intensive developments in plant protection products, is still a field that requires new and more efficient methods, which can improve crop yields. One of the biggest challenges in agriculture is the prevention of plant diseases caused by viruses, bacteria, fungi, etc. It is well known that there are many chemical compounds that possess biological activity, such as antibacterial (e.g., the quaternary ammonium salt: didecyldimethylammonium chloride $[N_{111010}][Cl]$[1]) or antifungal (propiconazole and difeconazole[2]), and because of their properties, such compounds could find applications in plant protection.

Current methods for plant protection are usually limited to: (i) overall strengthening of the plants through fertilization, (ii) infection prevention, by controlling infected plants using compounds acting directly on pathogens (microbicides), (iii) elimination of unwanted plants (weeds) using herbicides.[3] Unfortunately, the prevention of viral infections in plants, due to the fact that viruses are non-living matter very closely associated with the host, is not possible using conventional plant protection agents; thus, the only possible way to prevent such infections is to increase the immunity of the plant against viruses. Among common methods used to immunize organisms against viruses are plant crossing, genetic modifications, or the recently discovered systemic acquired resistance (SAR) induction.

The SAR phenomenon allows the plant to induce self-resistance against many microorganisms.[4,5] The main active signaling substance in SAR is endogenous salicylic acid (SA).[6] This compound activates a signaling cascade leading to production of pathogenesis related (PR) protein in plants.[7] It has been shown that the SAR can persist in tobacco plants for about 20 days.[8] Studies on *Arabidopsis thaliana* have shown that acquired immunity is transferred to next generations as well.[9] It is known, that some chemicals (called 'plant activators') can induce resistance to the same spectrum of pathogens as in the biological model.[10] Such compounds have no antimicrobial activity on their own, but stimulate the resistance system of the plant to act against the pathogen. Well-known inducers of plant resistance include BABA (β-aminobutyric acid), AABA (α-aminobutyric acid), GABA (γ-aminobutyric acid), 2,6-dichloroisonicotinic acid, and salicylic acid.[11,12] Unfortunately, these chemicals are not used widely in the agrochemical industry. One compound which is commercially used is benzo[1,2,3]thiadiazole-7-carbothioic acid *S*-methyl ester (BTH). Its plant resistance-inducing properties have been widely described in the literature,[13–15] and this compound can be used for induction of resistance in certain plants such as tomato, corn, cucumber, beans, and tobacco. Due to their interesting mode of action, SAR inducers represent a unique group of compounds with the potential to become plant protection agents, that could be used in the fight

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against a very broad spectrum of pathogens (viruses, bacteria, and fungi at the same time).

Ionic liquids (ILs) are organic compounds comprising a cation and an anion which form a salt with a melting point below 100 °C. Through the wide choice of anions and cations, these compounds can be easily modified to obtain chemicals with adjusted physical, chemical, or biological properties.[16] In the literature there are many documented examples of ionic liquids adjusted to fit required properties. Some examples of anions commonly used in the preparation of hydrophobic ionic liquids include $[PF_6]^-$, $[BF_4]^-$ and $[NTf_2]^-$ [bis(trifluoromethane)sulfonamide].[17] There are also examples of using biologically active cations in the formation of ionic liquids, for example, antibacterial didecyldimethylammonium $[DDA]^+$ and benzalkonium $[BA]^+$ cations.[18]

Due to the presence of nitrogen atoms in the molecule of BTH it is possible to obtain its quaternary ammonium salts via alkylation, which can later undergo anion exchange in order to introduce an anion that possesses biological activity or modifies the physical properties of the obtained cationic BTH.

In this Letter we report the first synthesis and determination of the biological activity of ILs based on cationic derivatives of BTH. Our aim was to modify the physical and biological properties of neutral BTH by derivatizing it to a salt while maintaining its plant resistance inducing properties.

Sodium docusate [Na][Doc], the sodium salt of a strong sulfonic acid, consists of two parts: a long organic chain having nonpolar character (lipophilic), and a polar $-SO_3^-$ group having hydrophilic character. Such a chemical structure allows the formation of micelles (Fig. 1). For this reason, aqueous sodium docusate is a surfactant which reduces surface tension and therefore can facilitate the wetting of a variety of surfaces and provide a greater contact surface. Thus, it is used in drugs acting on the gastrointestinal tract because it decreases the surface tension of gastrointestinal epithelium, facilitating the penetration of water into the stool. Moreover, as demonstrated by Kern, docusate also exhibits bactericidal properties.[19] In the present study, the docusate anion was used in order to create a bifunctional compound in which the docusate ion acts as an antibacterial component, and increases the solubility in water of the resulting IL in comparison to the starting material, neutral BTH.

On the other hand, the $[NTf_2]^-$ anion is frequently used in the synthesis of ionic liquids in order to modify the physical properties of the resulting salts, especially water solubility.[20] Pairing cations with an $[NTf_2]^-$ anion often lead to sparingly water soluble products. In view of the fact that the solubility of neutral BTH is very low (only 7 mg/L), conversion of BTH into a cation results in a significant increase in its solubility in water, thus by using $[NTf_2]^-$, we can moderate this solubility. It is expected that the hydrophobic form of the BTH salt will remain on the surface of the plant for a longer period of time before being washed out in comparison with well-soluble BTH salts with a hydrophilic anion.

Ammonium salts such as $[BTHMe][MeSO_4]$ and [BTHMe][OTf] (Fig. 2) were synthesized using strong alkylation reagents: $Me_2SO_4$ (dimethyl sulfate) and MeOTf (methyl triflate). Following their synthesis, the alkylated BTH derivatives were subjected to anion exchange reactions in order to form [BTHMe][Doc] and $[BTHMe][NTf_2]$ salts (Fig. 2). Additional details on the synthetic protocols of the presented compounds are included in the Supplementary information.

The thermal properties of the reported compounds were determined using DSC and TGA analyses (Table 1). Each of the methylated derivatives of BTH exhibited a reduced melting point in comparison to neutral BTH. The most prominent reductions in the melting points were observed for [BTHMe][Doc], which is a liquid at room temperature, and $[BTHMe][NTf_2]$ which melts at 85 °C. Only $[BTHMe][MeSO_4]$ had a melting point above 100 °C (108 °C), but still lower than the melting point of the starting material, neutral BTH. Literature data indicate that compounds based on the $[NTf_2]^-$ anion, in general, show high thermal stabilities.[21] For example didecyldimethylammonium and benzalkonium cations with $[NTf_2]^-$ show thermal stabilities about 150 °C higher than in a case when the anion is nitrate. As observed by TGA, $[BTHMe][NTf_2]$ was stable to 199 °C (highest of all the compounds), while the other compounds showed a similar thermal stability to the starting material (144 °C for $[BTHMe][MeSO_4]$ and 134 °C for [BTHMe][Doc]).

In order to determine the dissolution rates of the salts obtained, and compare the results with the dissolution rate for the starting material BTH, a continuous dissolution system was assembled that allowed for a constant measurement of the absorbance of the solution by the UV–vis method. During sample dissolution, the UV–vis spectrum was recorded at a characteristic wavelength (338 nm) for BTH and $[BTHMe][NTf_2]$ and 316 nm for [BTHMe][Doc]. All experiments were performed at a constant temperature of 20 °C. In order to perform the experiment, a sample of the test substance, of known weight, was placed in a conical flask filled with a known amount of water and stirred with a magnetic stirrer (250 rpm/min). At the same time, from the top of the flask, a continuous flow of solution was taken (using a peristaltic pump) and transported through the UV cell. From the UV–vis apparatus outlet the solution flowed back into the vessel where the test substance was dissolved to form a closed, continuous system with a capacity of 160 ml. The flow rate was set at 8 ml/min using a peristaltic pump. The experiment was carried out each time to completely dissolve the test substance. The results of the experiments are presented in the form of a graph of absorbance versus time, where the absorbances of the individual compounds are normalized so that 100% of the absorbance is that at complete dissolution of the tested substance.

The above experiments were performed using BTH, $[BTHMe][NTf_2]$, and [BTHMe][Doc] (6–20 mg of substance in 140 ml) in order to evaluate the dissolution rates of these

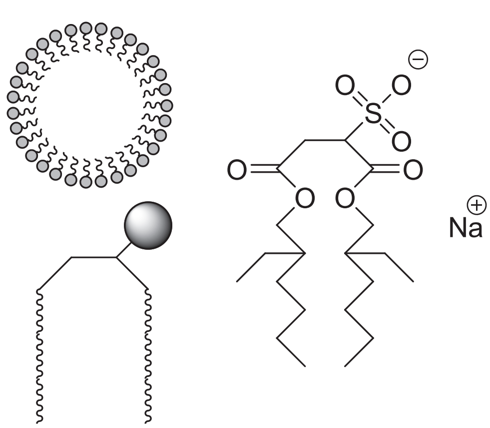


**Figure 1.** Sodium docusate is a surfactant that can exist in micellar form in aqueous solution.

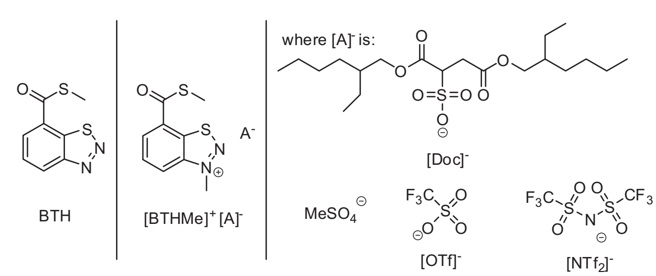


**Figure 2.** Structures of BTH and its ionic derivatives.