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Tesi

Versatility of Deep Eutectic Solvents (DESs): Green Restoration and Eco-sustainable Organic Synthesis

Settore Scientifico Disciplinare CHIM/06

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Abstract

Deep Eutectic Solvents (DESs) represent a class of unconventional solvents obtained from the mixture of two or more pure compounds, resulting in a liquid form at room temperature due to their lower melting point than those predicted for an ideal liquid mixture.

They stand out from conventional solvents due to their environmental sustainability, biodegradability, and versatility, attracting attention in various fields.

However, their potential application in cultural heritage restoration remains largely unexplored, given the limited number of scientific papers addressing the use of DESs in this field. This research project aims to prepare DESs and evaluate their efficacy as eco-friendly strategies for restoration practices. A first case study demonstrates the promising utility of DESs as green biocidal solvents for use in the removal of biofilms from artworks in the cultural heritage sector. A second case study highlights the ability of hydrophobic DESs, as cleaning agents, to remove aged non-polar coatings, addressing degradation products commonly encountered in restoration work on metal and stone assets. The search for new solvents, such as DESs, to replace harmful and unsafe conventional solvents used in the cultural heritage sector has shown promising results.

Furthermore, due to their unique characteristics, DESs can be used as unconventional solvents in organic synthesis representing excellent substitutes for volatile organic solvents. The main advantages of their use as reaction solvents are increased reaction speed, increased reaction yield and selectivity, and the possibility of recycling the solvent/catalyst system. DESs prove to be efficient solvents in the synthesis of high-value-added organic compounds, facilitating processes such as the iodocyclization reaction and the Sonogashira and Suzuki coupling reactions. This approach underscores the versatility and potential of DESs in cultural heritage conservation and cleaning, and in synthetic chemistry, offering a viable alternative with wide applicability.

Introduction

Since the first scientific paper was published on the emergence of the concept of Deep Eutectic Solvents (DESs)[1], which showed that the combination of two or more compounds - typically a hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) - could result in a mixture with amazing properties, the interest in the research of DESs and the study of their properties has grown significantly, especially with the expansion of their use in various fields.

The increasing amount of scientific literature on DESs applications has resulted in incorrect definitions of these solvents. Simply identifying a eutectic point, the formation of hydrogen bonding between HBA and HBD, and adhering to specific stoichiometric ratios between components do not adequately characterize a DES[2]. It is crucial to distinguish DESs from simple mixture or solutions.

A DES is defined as a eutectic mixture of two or more pure components for which the eutectic temperature is lower than that of an ideal liquid mixture. This temperature depression, ΔT_2, is the difference between the ideal eutectic point (T_E,ideal) and the actual eutectic point (T_E)[3]. Within composition ranges where they remain liquid at room temperature, they can be used as solvents.

The properties of this class of solvents depend on the chemical-physical characteristics, the molar ratio and the interactions between the components that define a DES[4].

The procedure for preparing DES is simple and inexpensive, starting with raw materials that are mainly of natural origin[5,6]. Once synthesized, no purification procedures are needed, and the atom economy is 100%[7], meaning all atoms from the starting materials are converted into the final product without generating any waste.

DESs exhibit unique chemical-physical properties, which have attracted the attention of the scientific and industrial community in recent years as eco-friendly alternatives to conventional solvents like Ionic Liquids (ILs) and Volatile Organic Compounds (VOCs). DESs are generally considered more environmentally benign due to their lower toxicity, higher biodegradability, and non-volatility compared to ILs and VOCs[8]. DESs typically have low vapor pressure, making them non-volatile compared to conventional solvents. Nonetheless, they generally exhibit lower thermal stability than ILs. However, not all DESs are non-toxic, necessitating efforts to evaluate and optimise their application for varying toxicity to humans[9]. Recent studies have revealed significant challenges regarding their actual sustainability, particularly in the case of Choline Chloride formulations[10]. The general claim of DESs as universally sustainable solvents, based only on their natural origins and the low toxicity profiles of their individual components, has been questioned. Issues such as antagonistic or synergistic effects among different components within DES formulations[11] call into question their overall eco-sustainability in the medium to long term.

Despite these considerations, ongoing fundamental research is essential to fully understand their properties and optimize their applications. DESs represent a promising class of solvents, offering significant benefits over traditional alternatives[12–14]. They found application in several research areas, including chemistry, biotechnology, biomedicine and the food industry.

In the chemical sector, DESs provide a viable alternative to traditional solvents, offering sustainable solutions for the synthesis of organic compounds and polymers. In addition to acting as solvents, DESs can also act as catalysts, demonstrating their effectiveness in a wide range of organic reactions. For example, DESs used as solvent-catalysts enhance the synthesis of heterocycles[15] by facilitating specific reactions with high selectivity and yield.

Deep eutectic mixtures possess high dissolution capacity, making them ideal for the extraction of bioactive compounds and metals. DESs are used to extract, for example, phenolic compounds[16], flavonoids[17], polysaccharides[18] from plant and food materials, achieving higher yields than conventional solvents. In addition, DESs have demonstrated the ability to selectively separate and recover various metals from a wide range of sources, including minerals, biological samples, soil, wastewater, e-waste, food and cosmetics[12,19].

In the biomedical field, DESs serve as solvents with antibacterial, antifungal, antiviral, and anticancer properties[20]. Researchers have also investigated their potential in enhancing the solubility, bioavailability, and efficacy of various drugs[21,22].

The search for DESs with antimicrobial properties in the biomedical sector parallels a growing need in the agro-food industry for new products with antibacterial and antioxidative qualities. Globally, food waste poses a significant challenge, resulting in the squandering of resources like water and money. To address this issue, eco-sustainable strategies can be implemented across the food chain. For instance, using non-toxic food preservatives[23] or recyclable food containers[24] with antibacterial and antioxidant properties represents viable solutions.

Based on the nature of constituents, their properties, and applications, DESs are classified into subcategories. Some of these subcategories are Natural Deep Eutectic Solvents (NADESs), Therapeutic Deep Eutectic Solvents (THEDESs), Polymerizable Deep Eutectic Solvents (PDESs), and Hydrophobic Deep Eutectic Solvents (HDESs). NADESs[25] are constituted of natural molecules, such as sugars, amino acids, and organic acids. Due to their natural origin, high biodegradability, and biocompatibility, they are used in various sectors. THEDESs[26] have significant pharmacological properties and they are specifically designed for use in the biomedical sector. PDES[27] based on polymerizable components, is used to realize polymeric materials with important characteristics, such as low viscosity and good thermal stability. To conclude, HDESs[28] are characterized by low solubility in water, and they are used in processes of extracting nonpolar organic and inorganic molecules. The division of DESs into different categories has helped simplify the recognition of these mixtures' different properties and functions.

Based on the unique characteristics of DESs described above, my current research project focuses on two main applications. First, the preparation of DESs targeted for application in the field of restoration, with the primary goal of optimizing formulations for cleaning and conservation of artworks of cultural interest. Second, these DESs are used as solvents in specific organic synthesis reactions to obtain innovative molecules through an environmentally sustainable methodological approach.

In the course of this research, DESs were evaluated by other research groups in the field of cultural heritage. In particular, menthol-based DESs were investigated as substitutes for traditional toxic solvents in the removal of natural varnish layers, including dammar and mastic, from paintings[29]. Furthermore, an interesting study explored the application of DES Choline Chloride-Urea combined with an EtOH-H_2O agar gel for the removal of thin layers of protein-based varnish from oil and egg tempera paintings[30].

These studies represent the few articles in the literature that highlight the use of DESs in the restoration sector. Recently, the analysis of DES as a new strategy in cultural heritage cleaning and conservation has marked a significant advance in the field, opening a new chapter in research.

Cultural heritage is an invaluable wealth composed of many assets that require constant conservation and restoration. From antiquities to modern artistic masterpieces and architectural works, these treasures are exposed to multiple risks[31], including chemical, physical, and microbiological damage[32], mainly due to the complex interactions between materials and the surrounding environment. Experts engaged in conservation and restoration face significant challenges daily, dealing with multiple hazards that can compromise the integrity of cultural property[33].

However, the use of methods and reagents that may not be in line with contemporary advances persists in cultural heritage conservation practices, potentially neglecting critical considerations such as operator safety and environmental compatibility[34–36]. The implementation of regulations such as REACH and the "GLOBAL HARMONIZED SYSTEM" has ushered in a new direction that promotes the adoption of innovative products designed with key parameters, such as low toxicity, safety from potentially explosive substances, assurance of hygiene, sourcing from renewable sources, and eco-sustainable criteria selected by consumers[37]. This regulatory evolution will soon influence the cultural heritage sector, moving it toward greener alternatives in terms of both products and adopted procedures[38].

In the cultural heritage field, biological degradation represents a significant question[39]. This process results from the proliferation and colonization of a wide range of macro and microorganisms, including bacteria, fungi, lichens, and other organisms, which contribute to the formation of biofilms. These biological layers, known as biological patinas or biofilms, accumulate on the surfaces of artworks, becoming a major factor in biodeterioration[40]. The combined action of biofilms along with other damaging agents, such as atmospheric factors and environmental pollutants[41,42], accelerates the degradation process of material surfaces. Additionally, artworks exhibited outdoors are notably more susceptible to biodeterioration due to prolonged exposure to environmental elements[43]. This deterioration can compromise not only the aesthetic appearance of the artwork but also its structural integrity and chemical composition. Biological patinas can be removed by mechanical, physical, biological method, or chemical methods[44]. Each method has distinct advantages and disadvantages, requiring evaluation by the operator based on the specific methodology, type of patina, and desired removal objective.

Mechanical methods (use of brushes, trowels, scrapers) are inexpensive and provide immediate results. They usually show limited long-term effectiveness, and the mechanical action produces particles that pose a potential health risk to operators.

Physical methods, such as the use of microwaves, ultraviolet (UV) radiation, laser cleaning and heat shock, are effective but require experienced personnel and expensive instrumentation. In addition, treatment times can be prolonged because the process often needs to be repeated several times to achieve the desired results.

Biological methods include the use of natural products, enzymes, and microorganisms or viruses as biocides. Although they offer clear advantages in terms of safety and environmental sustainability, it is important to conduct further research to evaluate their success over time and compare costs with traditional or chemical methods.

Chemical method consists of the use of biocides, organic and inorganic chemical compounds, which are a significant part of the techniques used in the restoration of artifacts. These substances can eliminate organisms, facilitating their removal by mechanical means. Despite their use in combating biological patinas, concerns arise regarding their harmfulness to humans, the environment, and treated materials. These compounds range from organochlorine biocides to organophosphorus pesticides and quaternary ammonium salts, each with specific persistence and toxicity characteristics [45,46]. Their short duration of action also requires frequent treatments, increasing the risks associated with their use.

To face the presence and growth of these biological patinas, the application of biocides is a common strategy. However, in the context of cultural heritage conservation, the choice and use of biocides are subject to strict constraints and specific requirements.

A crucial challenge for restorers is to identify a biocide that can act effectively on the layer to be treated without compromising the underlying layer that is an integral part of the cultural asset, using it at low concentrations, avoiding the formation of residues harmful to humans, the environment, and the artwork itself.

In recent years, the cultural heritage sector has shown a growing interest in adopting new strategies as alternatives to traditional methods. This interest is driven by the search for sustainable and safe solutions for the conservation and cleaning of cultural heritage.

The different categories of DESs share characteristics that have attracted the interest of researchers, in particular their antimicrobial properties[20]. Their antimicrobial properties have been examined and confirmed through various tests[47]. Although they have not yet been tested as biocides in restoration, DESs can be considered due to their widely documented properties, which also include antibacterial and antifungal action[48]. These green solvents could therefore be considered potential biocides, opening up interesting prospects for the industry in the future. Moreover, the protection and restoration of cultural assets is a constant debate, especially in the case of artworks exposed to the outdoor environment. It emphasizes the importance of safeguarding cultural artifacts not only during the restoration process, but also for their long-term preservation. This means protecting these assets from future damage and keeping their cultural significance intact.

Throughout history, objects of cultural value made of stone and metal, exposed to the effects of climate and air pollution, are inevitably subject to erosion and corrosion processes[49,50]. To preserve them, the use of non-polar protective coatings has often been adopted[51–53]. These coatings have traditionally acted as a 'sacrificial layer', to safeguard the artworks. Frequently used sacrificial layers are typically composed of beeswax or microcrystalline waxes.

Beeswax is renowned for its natural origin and relative softness. It forms a hydrophobic, low-gloss protective layer. However, beeswax tends to degrade over time due to its low melting point, incorporating dust and environmental pollutants[54].

Microcrystalline waxes are synthetic or refined waxes that have been processed to have smaller crystal structures than beeswax[55]. This characteristic makes them more resistant to temperature changes. Nevertheless, microcrystalline wax coatings also tend to degrade over time. During this time, they can exfoliate and turn to dust, compromising the aesthetic appearance of the artwork.

Over time, deterioration of these protective wax coatings becomes inevitable, resulting in accumulation of dust and contaminants, and alteration of the color of the artwork[56]. The necessary removal of these aged wax layers, which is essential to maintain the integrity of the works, currently relies on the widespread application of non-polar solvents[57]. Unfortunately, these solvents pose risks to the environment and human health because they are flammable and toxic[58,59].

Again, the focus is on discovering new green approaches such as cleaning treatments, for the removal of aged non-polar coatings.

In this context, DESs with hydrophobic characteristics emerge as possible candidates. DESs, having hydrophobic properties[60], could be very useful in removing aged wax layers while preserving the integrity of artworks. These solvents can be designed and adapted to meet specific conservation requirements, offering significant potential in the field of cleaning cultural artifacts.

After understanding the potential of DESs in removing aged wax layers, research focused on analyzing the interaction between beeswax and DESs. A preliminary theoretical study explored the effectiveness of various DESs in dispersing beeswax. In particular, Hansen solubility parameters have been calculated by the Hoftyzer-Van Krevelen method[61] and reported on the Teas chart[62]. The latter has been used to visualize solvent-solute interactions. Further theoretical analysis has been conducted by using the 3D solubility sphere method[63], which is based on the calculation of Relative Energy Difference (RED); the dispersing power of DESs increases when RED values are close to 0. The theoretical data allow for a classification of the dispersion power of the DESs under scrutiny. It is worth remarking that the two methods, Teas chart and RED, give different prediction for what concerns the dispersion power of the was in the DES. In order to corroborate the theoretical predictions, some experiments have been performed to test the dispersion power of DES and to build a phenomenological model of the dispersion process. In particular, both turbidity and the amount of beeswax dispersed in DES have been measured at different times. The experiment results are in agreement with the RED theoretical predictions while the Teas chart method turns out to be not reliable in this case.

Concerning the mathematical modelling, the work can be divided into three steps:

1. nonlinear regression of experimental data,
2. construction of a dynamical model,
3. interpretation of the model and development of the phenomenological model.

In order to build a regression model, the Software TableCurve 2D has been used to carry out data fitting. The LogLogistic regression model, $y = \frac{a}{1+\left(\frac{t}{b}\right)^{c}}$, resulted to be the best one in order to explain and to fit the data. In the formula above, the independent variable t is the time and y is the turbidity or the amount of dispersed beeswax.

In order to introduce a dynamical model corresponding to the dispersion phenomenon, we need to identify the differential equation of which $y = \frac{a}{1+\left(\frac{t}{b}\right)^c}$ is a solution. It is straightforward to see that it is

$$\frac{dy}{dt} = \frac{k}{a} y^{1+\frac{1}{c}} \left(1 - \frac{y}{a}\right)^{1-\frac{1}{c}}, \qquad b = \frac{c}{k}, \tag{1}$$

where the parameter a represents the saturation of the beeswax in the DESs since $y = a$ implies that the derivative $\frac{dy}{dt} = 0$ and then that the dispersion process stops.

Starting from (1), an interpretation of the model can be suggested. That will be the topic of a forthcoming paper.

Research studies conducted during this work show that DESs, due to their specific properties, present promising results for their biocidal action in removing biodegraded layers. In addition, DESs stand out for their success in removing aged wax layers. In both cases, they offer a new alternative to traditional solvents. Therefore, the use of DESs in the cleaning and conservation of cultural artifacts emerges as a green strategy that contributes to the preservation of the integrity and beauty of artworks.

Simultaneously, in the field of organic synthesis, DESs have revolutionized traditional approaches, emerging as highly efficient substitutes for conventional organic solvents[64]. The use of conventional solvents in organic synthesis reactions, although widely adopted, raises concerns about their toxicity, volatility and flammability[65]. The evolution of research that focuses on identifying alternative solvents, highlights DESs as promising solutions for replacing conventional solvents. The use of DESs as reaction solvents has been shown to improve reaction kinetics, yield and selectivity[66]. In addition, their use can enable recycling of the solvent/catalyst system, a key feature in a chemical process[67].

DESs are emerging as viable substitutes for traditional solvents in numerous organic synthesis reactions, overcoming many of their limitations and opening up new perspectives for synthetic chemistry. These innovative solvents can be used as both solvents and reagents, demonstrating exceptional versatility.

For example, DES are used as reagents in the synthesis of geminal diureas from aldehydes[68], in the synthesis of aminoxazole derivatives from phenoacyl bromides[69], and in multicomponent reactions (MCRs) enabling the synthesis of dihydropyrimidinone derivatives[70].

DESs demonstrate remarkable efficiency in redox reactions, achieving high conversion yields and selectivity, particularly for the oxidation of primary and secondary benzyl, allyl, and alkyl alcohols to carbonyl compounds[71]. They also show effectiveness in the oxidation of sulfides and the hydroxylation of arylboronic acids[72]. Additionally, they have been utilized for the fluorination of acetophenones, facilitating the one-pot synthesis of α-fluoroacetophenones[73].

DESs prove highly versatile in organic reactions, serving both as solvents and also as catalysts in a wide range of chemical transformations.

They facilitate processes such as esterification of carboxylic acids with alcohols, acetylation reactions, and the glycolysis of polyethylene terephthalate through transesterification with ethylene glycol[74-77]. In addition, DESs have been used in the formation of formanilides and diarylamidines from anilines and formic acid or trimethyl orthoformate[78].

These deep eutectic mixtures enable the synthesis of a wide range of heterocyclic derivatives, for example, xanthene derivatives[79], quinoline derivatives[80], imidazoles[81] and pyrroles[82]. They also facilitate the acid-free Paal-Knorr synthesis of pyrrole derivatives and, in the absence of amines, the formation of furans[83]. They also play an integral role in Pechmann condensation in the synthesis of coumarins[84].

Additional successful reactions conducted in DESs include conjugate additions such as thia-Michael additions[85]. A notable example of reaction is the DES-catalysed Henry reaction under microwave irradiation, where is used to facilitate the formation of (E)-nitroalkenes from aldehydes and nitroalkanes[86].

These are some examples of the use of DESs in chemical reactions. Of course, there are a variety of uses of DESs in the field of chemical synthesis in the literature. In conclusion, the innovative use of DESs in organic reactions is revolutionizing synthetic methodologies, offering recyclable and efficient solutions for the synthesis of complex molecules of pharmaceutical, application and industrial interest.

The current research demonstrates that ChCl-based DESs have been successfully used in iodocyclization reactions of 2-methylthiophenylacetylenes for the synthesis of various 3-iodobenzothiophenes. These compounds represent a class of derivatives of considerable pharmacological importance and can serve as precursors for the synthesis of more complex molecules. 3-iodobenzothiophenes have been used as substrates in Sonogashira and Suzuki coupling reactions, yielding further functionalized molecules. The versatility and effectiveness of DESs in these reactions make them a promising choice for the synthesis of molecules with pharmaceutical and chemical interest.

These deep eutectic solvents have proven effective in implementing new environmentally sustainable methods to obtain novel molecules with high yields, allowing the DES-catalyst system to be recycled without a significant loss of desired product yield. This translates into a significant time and economic benefit.

This innovative approach, conducted in DES, eliminates the use of toxic and flammable conventional solvents, known as volatile organic compounds (VOCs), minimizing the negative impact on operator health and the environment[87]. These promising results not only underline new strategies for the synthesis of variously functionalized compounds but also highlight the potential of the synthesized molecules for future pharmacological and application developments.

As seen above, the chemical-physical properties of DESs offer a wide range of applications, spanning both restoration and chemical synthesis. This emerging class of solvents opens the door to innovative and sustainable solutions.

The investigation of their potential as biocides and cleaning agents in the cultural heritage sector, and finally their role as solvents for the synthesis of organic molecules, are the crucial topics analyzed during the doctoral course.

Each of these aspects will constitute a dedicated chapter within this paper, thus providing a comprehensive overview of the different applications of DESs. In the following, each chapter will be explored in detail.

Chapter 1: This chapter explores the use of DESs as biocidal agents in the field of cultural heritage, with a focus on analyzing their effectiveness in removing biological patinas. Five different combinations of ChCl-based DESs were applied directly in situ, specifically on two areas of a Roman mosaic characterized by a biodegraded surface layer. To estimate the interaction of DESs with the material, adsorption tests were performed in laboratory, measuring electrical conductivity. To assess the biocidal efficacy of DESs, extensive analyses were conducted, including ultraviolet fluorescence, bioluminescence, and spectrocolorimetry. These analyses were conducted comparing the situation before and after the application of DESs, and also compared with the efficacy of a biocide widely used in the restoration field, Preventol RI50. This comparison allowed to evaluate the biocidal effects of DES compared with conventional biocide.

Chapter 2: This chapter focuses on analyzing the role of hydrophobic DESs in removing aged layers of waxes used as protective layers in the restoration field, examining in depth their ability to remove them safely and effectively. During this study, various HDESs, consisting of natural molecules such as thymol, menthol, and long-chain fatty acids, were tested in the laboratory, using slide samples with aged non-polar coatings. These green solvents were evaluated for their ability to remove three different types of waxes: beeswax and two types of microcrystalline waxe (R21 and Renaissance), commonly used in the past for the conservation of stone and metal artifacts. The interaction between each DES and each type of wax was evaluated using solubility tests, contact angle measurements, and cleaning tests. The results obtained were compared with those obtained from traditionally used solvents, such as Ligroin and White Spirit. DESs have been shown to be highly effective due to their chemical structures, which have an affinity for the specific chemical compositions of waxes. The results demonstrated the efficacy of specific HDESs in removing specific waxes.

Chapter 3: This chapter aims to examine the use of DESs as solvents in the synthesis processes of organic molecules. Initially, the use of DES Choline Chloride/Urea as solvent in the iodocyclization reaction of the substrate 2-methyl-4-(2-methylthio)phenyl)but-3-in-2-ol was examined, which allowed obtaining the desired product 2-(3-iodobenzo[*b*]thiophen-2-yl)propan-2-ol with an excellent isolated yield of 81%. Having identified the best reaction conditions, the generality of the process was evaluated by employing variously substituted starting substrates. The 3-iodobenzothiophene derivatives were obtained selectively and in excellent yields. It is important to note that, in some of these reactions, the DES/catalyst system was successfully recycled several times without a significant reduction in the yield of the desired product. Subsequently, the 3-iodobenzothiophene products were used as starting substrates in Sonogashira and Suzuki coupling reactions in DES. It is essential to emphasize the recyclability of the DES-catalyst system in these types of reactions as well. This approach has allowed us to develop a new green synthesis strategy, obtaining innovative molecules with a benzothiophene core, potentially relevant in the application and pharmacological fields. Through such reactions, it has been possible to achieve the synthesis of variously substituted molecules with a benzothiophene core, using an eco-sustainable and recyclable methodological approach.

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Chapter 1

Article: Deep Eutectic Solvents (DESs): Preliminary Results for Their Use Such as Biocides in the Building Cultural Heritage

Role and contributions of the candidate: In the present research, I was primarily responsible for the preparation of DESs, actively participating in the development of the methodology and execution of the experiments.

This article was published in Materials, volume 15, in 2022, p. 4005. Available at: https://doi.org/10.3390/ma15114005. Intitled "Deep Eutectic Solvents (DESs): Preliminary Results for Their Use Such as Biocides in the Building Cultural Heritage", the authors are Andrea Macchia [1,2], Romina Strangis [3], Sara De Angelis [2], Marica Cersosimo [2,3], Antonella Docci [4], Michela Ricca [1,*], Bartolo Gabriele [3], Raffaella Mancuso [3,*] and Mauro Francesco La Russa [1]

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Abstract: Biodeterioration is an increasingly widespread process of degradation in the context of the conservation of Cultural Heritage, which involves a combination of physical and chemical damages together with an aesthetic alteration of materials. For biological damage on monuments caused by pathogens, macro- and microorganisms, chemical treatments are generally used, most of the time dangerous for the environment and for the operator. In this context, new eco-friendly products represent necessary tools for the treatment of biologically deteriorated stone surfaces and represent a new challenge in the field of restoration and conservation of materials of cultural interest. A relatively new class of unconventional green solvents are deep eutectic solvents (DESs), which have peculiar chemical-physical characteristics such as being non-toxic, ecological, biodegradable, non-flammable, and stable in the presence of water. Furthermore, many DESs known in the literature have also been shown to have a biocidal action. All these characteristics make DESs very advantageous and safe, and they could be used as biocidal agents for the treatment of biodegraded surfaces of Cultural Heritage, being non-toxic for the environment and for the operator. So far, they are used in various fields, but they still represent a novel frontier in the Cultural Heritage sector. The present research aims at testing five different DESs for the first time in Cultural Heritage. In particular, DESs are applied to a mosaic located in the Ostia Antica Archaeological Park (Rome), and their efficiency is compared with a biocide product currently used in the restoration field, namely, Preventol RI50, through luminescence, bio-luminometry, and spectrocolorimetry analysis. The preliminary results achieved show the different behaviors of each DESs, highlighting the possibility of employing them in the field of Cultural Heritage. Further studies have been planned, some of which are already underway, to investigate the properties of DESs and indicate any improvements to make them more effective, both as solvents and as biocides, and easy to apply to various types of materials. The results obtained from this first study are very promising for the use of DES as a new green strategy for cleaning and conservation treatments of materials in the field of Cultural Heritage.

Keywords: DES; biocides; green conservation; Cultural Heritage; biodeterioration; solvent

1. Introduction

The problem of biological attack is increasingly widespread in the conservation of Cultural Heritage. This type of degradation is mainly found on works placed outdoors, but also indoors, and is due to the growth and accumulation of organisms, such as bacteria, algae, fungi, lichens, and mosses [1–3]. Depending on the levels of humidity, light, and nutrients, these organisms form complex and heterogeneous biostructures of biological patinas, which are called biofilms [4]. In this way, interactions between biofilm, substrate, and external environment are triggered, leading to the main biodeterioration processes, such as accumulation of dirt and particles, loss of material by contraction, and expansion, and so on.

The cleaning of surfaces from biodeterioration takes place through various mechanical, chemical, and physical systems so far. Generally, cleaning procedures include processes for removing or reducing layers that are deposited on the artwork over time, causing damage. The reason for their removal is due to the state of conservation of the material, which no longer adequately responds to its functionality or the aesthetic values, altering the perception of the artwork and sometimes causing irreparable damage. [3–5].

The most used method to clean surfaces from biological colonization is based on chemical substances, exploiting the action of biocidal substances [4]. These techniques are characterized by being harmful to the operator and the environment [5–9].

In the field of conservation of Cultural Heritage, the biocides used are not numerous as they must have specific requirements such as efficacy at low concentrations towards target organisms, non-interference with the constituent material of the artwork, and low risks for humans and the environment. Among the major compounds used by restorers to prevent the spread of biological patinas on stone materials, there are biocides based on quaternary ammonium salts (QASs), such as Preventol RI50. Their biocidal action is based on the destabilization of the cell membrane structure, leading to rapid cell lysis [10]. Although QASs are used at maximum concentrations of 5%, showing good results as biocides in various fields, they are dangerous for the operator and the environment. Indeed, many studies observed an abundant presence of QASs in soils caused by their rapid absorption and strong resistance to biodegradation in anoxic/anaerobic conditions

[11], as well as causing a reduction in the efficacy of biocides and an increased resistance to antibiotics in microbial communities over time [12].

Considering the aforementioned properties of commonly used products, there is an urgent need for new biocides for the Cultural Heritage field, which have a lower impact on the environment and the operator. In this regard, DESs (Deep Eutectic Solvents) shall be considered. DESs are non-toxic solvents having good biodegradability, high dissolution ability, non-flammability, chemical and thermal stability, low vapor pressure, low melting point, low-cost, and solvent-free preparation [13,14]. Generally, they are liquid at temperatures lower than 100 °C and can be prepared by mixing hydrogen bond donors (HBD) and acceptors (HBA) in a stoichiometric ratio [15]. The interactions between these components lead to a decrease in the melting point, caused by the delocalization of the charge through the hydrogen bond between the halide ion and the hydrogen donor part [15].

The most common components used for DESs were choline chloride (ChCl) or urea as HBA, and glucose or oxalic acid as HBD. Generally, DESs are identified as sustainable solvents displaying low toxicity. Indeed, they are eco-friendly compounds, with good stability in the presence of water, high biodegradability, and low toxicity [16–18].

Some mixtures of DESs also have a biocidal and inhibiting action against some bacterial strains, in particular, towards Gram-positives, which have a structure less complex than Gram-negatives and are therefore more easily attackable [16,19]. It should be considered that the antimicrobial properties of DESs depend on various factors, although the mechanisms are not yet clear. Indeed, in various studies, a different biocide response was found depending on the various components that make up the DESs, which is also different from the activity of the individual components. This could be due to the delocalization of charges that occurs during their formation. For example, one of the most commonly used salts in the preparation of DESs, ChCl, has a delocalized cation that favors greater interaction with the negative groups present on the surface of cell membranes, leading to distortion and ruptures of the cell wall. Therefore, it leads to increased toxicity against microorganisms. Another factor proposed to explain the toxicity of DESs is their acidity or alkalinity (pH). Indeed, it must be borne in mind that bacterial and fungal growth are favored by optimal pH ranges (6.5–7.5 and 5.0–9.0, respectively). At the same time, values that are far from optimal cause denaturation of the proteins found on the cell membrane of the microorganism, together with negative effects on cellular activity. Consequently, DESs with acidic pH will exhibit a more pronounced biocidal effect [16–18]. Moreover, in a few studies, some DESs have been used as antifungal products for eukaryotic microorganisms such as Phanerochaete chrysosporium, Aspergillus niger, Lentinus tigrinus, and Candida cylindracea, microorganisms also found on artifacts in the field of Cultural Heritage [16].

Due to these characteristics, DESs can be an alternative in the Cultural Heritage sector, as it is possible to combine and modulate them according to needs, varying the molar ratios of the components and the water content, thus enhancing their versatility.

For the first time, we propose, in this study, the use of specific DESs as new greener solvents with biocidal activity on a mosaic in the Archeological Park of Ostia Antica. DESs were evaluated and analyzed by luminescence, bioluminometry, and spectrocolorimetry in order to assess their biocidal capacity, comparing their performance with the results obtained from the application of Preventol RI50.

The DESs used in the present work (ChCl/Eg, ChCl/MalAc, ChCl/Gly, ChCl/OxAc, and ChCl/U) are synthesized from natural precursors that are present in nature, in plants, or as secondary metabolites in humans and animals. From studies reported in the literature, they appear to be environmentally friendly and safe for humans. For instance, choline chloride, which is the HBD component for all DESs studied, is a very safe chemical molecule. Indeed, choline, its precursor, is an essential substance for human beings [20]. Malonic acid is a compound found in natural biological systems, such as legumes, and plays an important role in the development of animal and human brains [21]. Oxalic acid and its salts are end products of the metabolism of many types of plants and are used in conjunction with ascorbic acid for their positive impact on delaying browning and maintaining the overall quality of litchi fruit [22]. Urea is a naturally occurring metabolite that is expelled by humans. Some studies have shown that it can inhibit the proliferation of tumor cells [23], and glycerol is a triol of natural origin too, widely used in pharmacology and, as it is well known, in the preparation of beauty products due to its high-power emollient. Etylen Glycol, on the other hand, is used as a raw material in the manufacture of polyester fibers and for antifreeze formulations, and in poly(ethy1ene glycols) (PEGS) for some pharmacological applications [24].

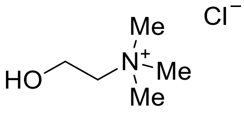
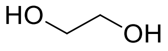
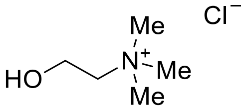
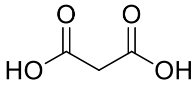
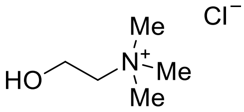
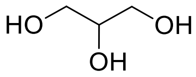
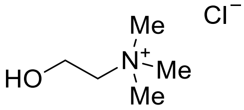
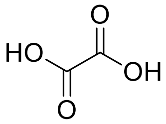
2. Materials and Methods

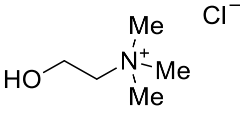
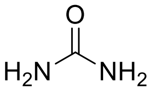
2.1 DESs Preparation

For the synthesis of DESs, Choline Chloride, Ethylene Glycol, Malonic Acid, Glycerol, Oxalic Acid, and Urea were purchased from Sigma Aldrich (Merck KGaA, Darmstadt, Germany).

DESs were synthesized by weighing and mixing, at the proper molar ratio, the HBD and HBA molecules and by mixing and heating them (80–100 °C) until homogeneous liquids were formed in times spanning from 1 to 3 h. The chemical structures of solvents' components and their molar ratios are reported in Table 1. The pH value of each DES is also reported in Table 1.

Table 1. Composition and pH of DES studied in this paper

DES code	DES Composition	HBD	HBA	Molar ratio (HBD:HBA)	pH
DES 1	ChCl/Eg	 Choline Chloride	 Ethylene glycol	1:2	5.6 ± 0.5
DES 2	ChCl/MalAc	 Choline Chloride	 Malonic Acid	1:1	2.8 ± 0.5
DES 3	ChCl/Gly	 Choline Chloride	 Glycerol	1:2	5.3 ± 0.5
DES 4	ChCl/OxAc			1:1	3.2 ± 0.5

		Choline Chloride	Oxalic Acid		
DES 5	ChCl/U	 Choline Chloride	 Urea	1:2	7.2 ± 0.5

DESs, as already mentioned, are a eutectic mixture, which occurs in the liquid state following the union of two solid components. The DESs' pH is due to the solvation water, present in very little amounts, which is closely anchored to the DESs. The nature of DESs is such that the two components, even if acidic, cannot release the proton, because they are strictly interconnected by an intrinsic donor-acceptor relationship. Only in aqueous solution, with a dilution greater than 50% (*v/v*) [25], would the eutectic nature, of the mixture, be destroyed and the acid component of DESs would be given the possibility to act as an acid species. Since DESs are used pure in the present work, and not in aqueous solution, they cannot release protons and therefore cannot interact with the stone layer but can only carry out antibacterial activity on the biofilm.

2.2 Experimental Set and Analytical Methods

Two areas of a mosaic flooring in the Archeological Park of Ostia Antica were selected as sample areas to conduct the experimentation (Area 1, Area 2), further subdivided into nine zones. In this study, only two zones (Figure 1) related to the application of Preventol RI50 (framed in red) and DESs (framed in green) will be considered. One area was left untreated and used as a reference (framed in yellow).

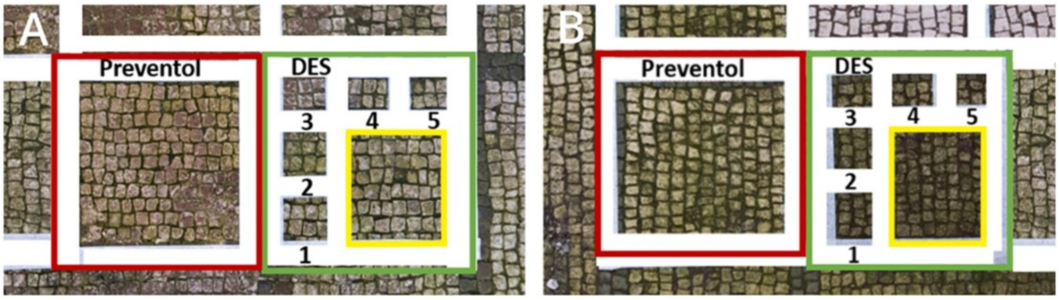


Figure 1. Two selected sample-areas of the mosaic in the Archeological Park of Ostia Antica: Area 1 (**A**) and Area 2 (**B**). The areas provided for the treatment with DESs (code 1–5) have been indicated with the green box, the one with Preventol Rl50 in red, and the untreated area in yellow.

The products were applied by brush, creating an even layer. DESs were used “pure”, without diluting them in organic solvents or water. The experimentation was conducted in September 2021 with the following microclimate: 24 ± 2 °C and 61 ± 5% of UR%. They were left to act for 3 days, and then they were removed with a mechanical action by brushing and washing.

A bioluminometer (KAIROSafe PD30) was used to determine the biological contamination of the surfaces treated with DES products and Preventol RI50. For this purpose, was used the wipe method, repeating the test three times which allowed subsequent statistical analysis [26]. The analysis is based on the phenomenon of bioluminescence, which is the ability of living organisms to emit light through enzymatic reactions, converting chemical energy into light energy. More specifically, this phenomenon is determined by the reaction that occurs between the luciferase enzyme, the luciferin substrate, and the adenosine triphosphate (ATP) molecule, used by animal and plant cells to accumulate, and exchange energy. Consequently, the amount of light emitted will be directly proportional to the amount of ATP present in the sample, expressed by the Relative Light Units value (RLU), thus providing an assessment of the overall levels of organic understanding of the surface under consideration. It should consider that damaged cells or dead cells will no longer be able to produce ATP.

To investigate the distribution of biofilms on the surface before and after treatment, ultraviolet fluorescence imaging was acquired using a Madatec multispectral imaging system, in order to capture the visible light emitted by the microorganisms still present after the application of the studied biocides.

The evaluation of the chromatic variation was observed using a spectrocolorimeter (Y3060 3nh, Shenzhen 3nh Technology Co., Ltd., Shenzhen, China) in the CIE L* a* b* space, where the L* coordinate represents the brightness, a* and b* represent the chromaticity coordinates, on the green/red axis and on the blue/yellow axis, respectively.

Then, chromatic alterations were analyzed by studying the ΔE, defined by the following equation:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Given the differences between the chromatic parameters of the surface before and after the treatment (ΔL^*, Δa^*, Δb^*). These measurements allow us to monitor bacterial growth on the surface under examination, as negative ΔL^* and Δa^* and positive Δb^* values indicate a bacterial recolonization process, while for ΔL^* and Δa^* values positive and negative Δb^* there will be bacterial decolonization of the surface, as well as biocidal efficacy of the analyzed solvents [27]. The measurements were repeated three times to reduce the uncertainty of the analysis.

The interaction between DESs and the substrate (mosaic tesserae) was studied in laboratory through electrical conductivity measurements using a low porosity stone (marble samples) and the most soluble DES used in the experimentation, as follows: Choline Chloride: Urea (DES 5). Soluble salts are the main degradation problem in porous stone materials, due to the formation of efflorescence (sub- and surface-).

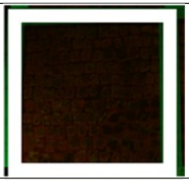
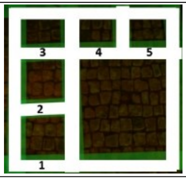
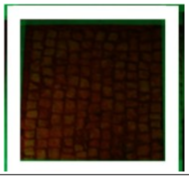
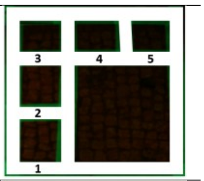
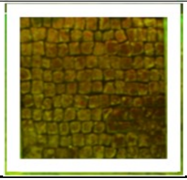
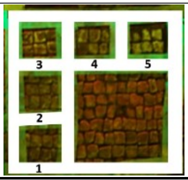
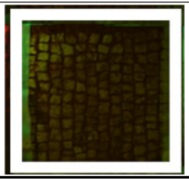
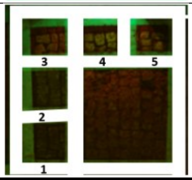
Electrical conductivity measurements were carried out to study the release of chlorine ions in the stone substrate, thus assessing the solvent's interference with the constituent materials of the artwork. A HD2156.2-Delta OHM (Delta OHM S.r.l., Caselle di Selvazzano, Italy) was used for analysis. In total, 700 mg of DES was applied to the marble sample with a steel spatula. The marble sample was placed in an Angelantoni climate chamber for 24 h at the following different temperatures: 20, 40, 60 °C, and 70% U.R. These parameters were defined based on the temperature and humidity of Ostia Antica during the experimentation time as follows: 24 ± 2 °C and 61 ± 5% of UR%.

After 24 h the DES was removed from the marble sample. The marble sample was left in 400 mL of deionized water (EC = 7 ± 2 µS/cm) for 3 h. The electrical conductivity was measured and referred to the concentration of totally dissolved DES in 400 mL of deionized water (750 ± 5.6 µS/cm). The test was repeated three times to reduce the uncertainty of the analysis.

3. Results and Discussion

From ultraviolet fluorescence analysis (Table 2), it is possible to distinguish the distribution of the microorganisms present in the two areas of the pavement through the light that is re-emitted by the chlorophyll molecules constituting the microorganisms when these are affected by UV radiation. In the two areas, before treatment, red fluorescence is observed, which is faded after the application of the biocides, following the devitalization of the photosynthetic microorganisms.

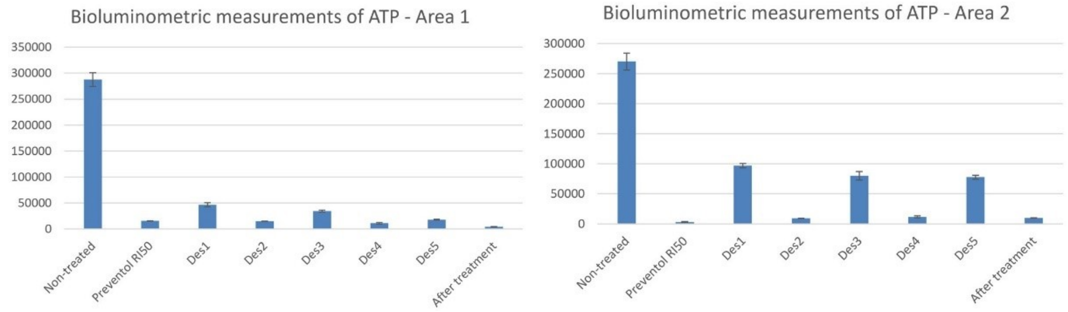
Table 2. Ultraviolet fluorescence imaging of Areas 1 and 2, before and after treatment with DESs and Preventol RI50.

	Area 1		**Area 2**	
	Preventol RI50	**DES**	**Preventol RI50**	**DES**
Before the treatment				
After the treatment				

In Area 1, a progressive biocidal response is observed for DES 5, 4, and 2, followed by DES 3 and 1; in Area 2, for DES 4, 2, and 5, followed by DES 3 and 1. This response is in agreement with the literature [28]. Indeed, DES 2 and 4, consisting of malonic acid and oxalic acid, respectively, have a more acidic pH, therefore creating conditions that limit bacterial growth.

Despite the good performance obtained, DES does not reach the standards obtained by using Preventol RI50 (Köln, Germany).

Anyway, it is extremely important to underline that the DESs studied in the present work, compared to Preventol RL50, commonly used in the field of Cultural Heritage, are stable, non-volatile, environmentally friendly, and do not require solvents for their dilution. Indeed, they can be applied pure because they are liquids or slightly viscous at room temperature. DESs are characterized by the mixing of two natural components that are not harmful to humans and therefore to the operator who applies the product to the stone artifact.



The bioluminometric measurements for Area 1 and Area 2 are shown in Figure 2. The values of RLU and standard deviation (SD) are reported, indicating the average and the SD of the different areas, comparing the non-treated and treated areas. Consequently, a good biocidal action is represented by low values reported in Figure 2, indicating a lower production of ATP due to the death or damage of the cells of the microorganisms capable of producing it.

Figure 2. Bioluminometric measurements of ATP of the areas of interest. The values of RLU and SD are reported for Area 1 and Area 2 comparing them to the non-treated and the mechanically and chemically treated areas.

From this, the best results were obtained for Area 1 and Area 2 by DES 2, DES 4, and by Preventol RI50, which show the lowest residual biological activity.

Table 3 shows the variations of the colorimetric parameters (L*, a*, b*) after the application and removal by mechanical cleaning of the biocides tested in the two areas, calculated as the geometric distance that separates them from the colorimetric parameters L*, a*, b* of the same areas before treatment.

Table 3. Colorimetric parameters (ΔL*, Δa*, Δb*, and ΔE) of Area 1 and Area 2.

			Area 1				**Area 2**	
Biocides	**ΔL***	**Δa***	**Δb***	**ΔE**	**ΔL***	**Δa***	**Δb***	**ΔE**
Preventol RI50	−3.96	2.05	0.15	4.46	0.18	2.78	−6.17	6.77
DES 1	0.15	−1.13	−9.63	9.70	−4.01	0.96	−2.64	4.89
DES 2	6.78	−1.97	−7.52	10.32	−0.29	0.55	−6.96	6.99
DES 3	5.03	−0.67	−1.81	5.39	−2.89	0.88	−4.93	5.78

DES 4	9.37	−1.52	−6.75	11.65	−0.63	2.85	−3.86	4.84
DES 5	1.69	−0.34	−1.73	2.44	6.62	1.40	−2.17	7.10

The average values of the color parameters L*, a*, b* before treatment in Area 1 correspond to L* 41.01; a* 2.56; b* 19.87; for Area 2, there are average values of L* 45.87; a* 0.78; b* 23.61. For Area 1, positive ΔL* values and negative Δa* values are observed for each area treated by the DES, while for Preventol Rl50, there is a negative ΔL* value (−3.96) and Δa* positive (2.05). As regards Δb*, there are negative values, except for Preventol Rl50 (0.15). This indicates that the areas following the treatment show a greening (Δa* negative), although not perceptible as the decrease in terms of absolute value is minimal.

The increase in brightness can be a consequence of the removal of the biological patina, which allows the color of the mosaic tiles to re-emerge. In contrast to Area 1, Area 2 shows negative ΔL* values for most DES, except for DES 5 (6.62) and Preventol Rl50 (0.18). This decrease, although minimal, causes a slight graying of the surface. Positive and negative Δa* and Δb* values are observed for each treated area, indicating less greening and yellowing. Therefore, it is possible to state that the best results are obtained from the application of DES 2 and 4 for Area 1, presenting an increase in the L* parameter and a decrease in the a* and b* parameters; of DES 5 for Area 2 since ΔL* and Δa* increase, while parameter b* decreases, thus leading to less yellowing.

Figure 3 shows the full spectrum color measurements of the treated and untreated areas (indicated by a black curve). From the spectra, it is possible to observe an absorption of around 670 nm, relating to the absorption of chlorophyll, for most of the biocides applied. However, this trend is especially decreased in both the areas where Preventol T150 was applied and, to a lesser extent, in the areas treated with DES 2 and 5 (Area 1), indicating the reduction of living microorganisms. Indeed, photosynthetic biodeteriogens lose chlorophyll when they die—together with the green color—thus enabling the evaluation of the treatment's efficacy by examining the typical absorption at 670 nm induced by ultraviolet light (UV) at 365 nm. A reduction in absorption reflects a reduction in microorganisms' vitality.

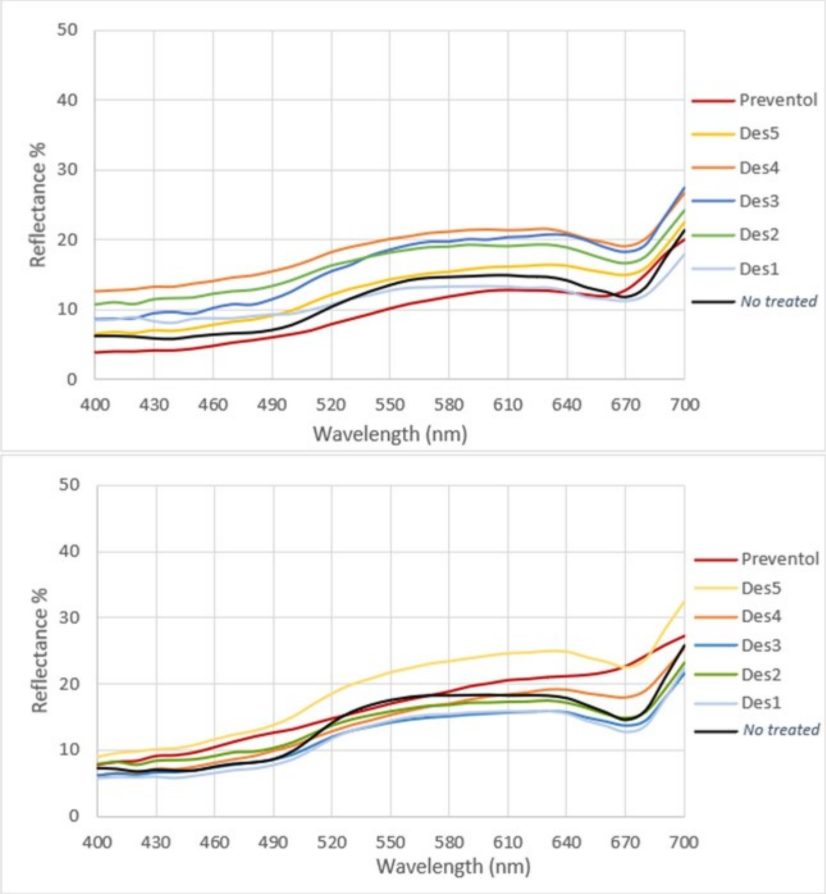


Figure 3. Full-spectrum color measurement of the treated zone in Area 1 (**above**) and in Area 2 (**below**) in comparison with the no treated zone indicated in black.

Electrical conductivity tests allowed us to define the capillary absorption of the DES by the porosity of marble using the ratio between the EC measured on the DES residue present in the sample and the EC of the totally dissolved DES in water and the relative error (expressed in %). A 100% ratio means that all the amount of DES was absorbed by the sample. The results can be seen in Table 4.

Table 4. Results obtained from electrical conductivity measurements (EC) on laboratory samples treated with Choline-Chloride:Urea.

Amount of applied DES (mg)	**Temperature (C°)**	**Amount of residual DES in the specimens (mg)**	**EC (μS/cm)**	**Corresponding DES in water (%)**
523 ± 5	60	304 ± 2	414 ± 6.4	55%

512 ± 7	40	55 ± 2	73 ± 5.7	9%
520 ± 4	25	34 ± 5	41 ± 5.3	6%

The test defined a low capillary absorption of DES by the substrate in the microclimatic conditions that characterized the archaeological site of Ostia Antica during the experimentation. The absorption of chlorine by the stone substrate increases as a function of the temperature due to the deliquescence of the salts used as precursors for the formation of the DES.

4. Conclusions

Currently, the interest in DESs is increasing due to their specific characteristics, especially for their simple preparation, low cost, and versatility in various fields. In this study, for the first time, their biocidal action in the field of Cultural Heritage was tested as an alternative to traditional biocides, which are more dangerous for the environment and the operator. Five DESs with different compositions based on choline chloride with ethylene glycol, malonic acid, glycerol, oxalic acid, and urea were tested and compared with the traditional biocide Preventol RL50. The latter was applied in-situ on a mosaic in the Ostia Antica Archaeological Park. The treatment involved the application of the biocidal products (a water solution of Preventol RL50 and pure DESs) and the removal of the biological patina by mechanical action. To evaluate the efficacy of the products and the best treatment with DESs, the following various analytical investigations were used: ULV imaging, spectrocolorimeter, and bioluminometry. From the analysis carried out, the best results among the deep eutectic solvents studied were obtained by DES 2, 4, and 5, although their biocidal action is lower than that of the traditional Preventol Rl50. It was possible to observe a greater biocidal effect for acid-based DESs (malonic acid = DES 2; oxalic acid = DES 4) as they have a pH that does not fall within the ranges favorable to bacterial growth. The DES 5 (ChCl/U) showed good efficacy at a more basic pH (7.2 ± 0.5). DES studied in the present work, compared to Preventol RL50, commonly used in the field of Cultural Heritage, are stable, non-volatile, environmentally friendly, and do not require solvents for their dilution because they can be applied pure because they are liquids and slightly vis- cous at room temperature. DESs are characterized by the mixing of two natural components that are not harmful to humans and, therefore, to the operator who applies the product to the stone artifact. Following the recent introduction of these green solvents in the field of Cultural Heritage, their effectiveness over time has not yet been clarified, but from the preliminary data obtained in the present work, they could be promising and very per- forming in the field of Cultural Heritage. Future experiments can therefore be oriented in this direction.

Author Contributions: Conceptualization, M.F.L.R., R.M., B.G. and A.M.; methodology, R.S., M.C., S.D.A. and A.M.; software, A.D. and A.M.; validation, M.F.L.R. and R.M.; formal analysis, R.S., M.C., S.D.A. and A.M.; investigation, R.S., M.C., S.D.A., A.M. and A.D.; resources, M.F.L.R., R.M. and A.M.; data curation, A.M. and A.D.; writing—original draft preparation, S.D.A., M.R., M.F.L.R. and R.M.; writing—review and editing, M.R. and R.M.; visualization, M.F.L.R., R.M. and A.M.; supervision, M.F.L.R., R.M. and A.M.; project administration, M.F.L.R. and R.M.; funding acquisition, M.F.L.R. and R.M. All authors have read and agreed to the published version of the manuscript.

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Chapter 2

Article: Sustainable solutions for removing aged wax-based coatings from Cultural Heritage: exploiting hydrophobic deep eutectic solvents (DESs)

Role and contributions of the candidate: In this research, my main contribution is resource-related. Specifically, I conducted a comprehensive literature review to identify hydrophobic DESs suitable for the experiment. After selecting the appropriate DESs, they were prepared for use in the experiment.

This article was published in New J. Chem., volume 47, in 2023, pp. 5991-6000. Available at: https://doi.org/10.1039/D3NJ00228D. Intitled "Sustainable solutions for removing aged wax- based coatings from Cultural Heritage: exploiting hydrophobic deep eutectic solvents (DESs)", the authors are Chiara Biribicchi,*[ab]Andrea Macchia,[bc] Gabriele Favero,[d] Romina Strangis,[e] Bartolo Gabriele,[e] Raffaella Mancuso[e] and Mauro Francesco La Russa[c]

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Abstract: This study describes the investigation on the use of hydrophobic Deep Eutectic Solvents (DESs) for the removal of nonpolar coatings from works of art to replace toxic solvents. Beeswax and two microcrystalline waxes (R21 and Renaissance®) have been selected as reference nonpolar coatings since they are commonly present in their aged state on metal and stone artifacts. The interaction between the DESs and three waxes has been evaluated through contact angle measurements, solubility tests, and cleaning tests carried out by implementing a method that is ordinarily used by restorers. Tests have been conducted on mockups consisting of microscope glass slides covered by wax. The effective removal of the wax-based coating from the mockups has been assessed through spectrocolorimetry and multispectral imaging under visible (VIS) and ultraviolet light (UV) at 365 nm by loading the waxes with a fluorescent marker (Rhodamine 6G). Fourier Transform Infrared (FT-IR) spectroscopy in the Attenuated Total Reflectance (ATR) mode was performed to assess the presence of both the wax and the solvent on the swabs used for the cleaning tests, confirming the actual interaction among the solvent and the solute. The experimental process proved DESs' potential of being used as green solvents for cleaning treatments on Cultural Heritage.

1. Introduction

Wax-based protective coatings have been traditionally used to protect both stone and metal sculptures from the erosive action of weather and the corrosive action of rain and atmospheric pollutants such as SO_x, NO_x, CO_2, and chlorides.[1–3] The hydrophobic feature of these materials is one of the main reasons for their persistent use on outdoor metal and stone artworks. Indeed, outdoor bronzes, iron, and lead sculptures undergo corrosion processes that are essentially caused by the presence of water on the artwork's surface resulting from precipitation or condensation and by the washing effect of rainwater.[4–7] On the other hand, the growth of salt crystals within pores is one of the major damage factors in stone weathering and is driven or enhanced by water.[8] Waxes have low water vapor permeability and low gloss, which makes them ideal protective coatings.[9] Formerly, beeswax used to be applied on outdoor stone and metal artifacts.[1,2,7,10,11] The so-called ‘‘waxing’’ process represented a common practice in ancient restoration treatments that allowed for the protection of the artwork from rainfall and atmospheric pollution and sometimes for the toning of the surface.[2] Nowadays, stone and metal sculptures are usually treated with microcrystalline-wax-based protective coatings.[12–14] When it comes to metal artifacts, the coating is typically applied on an acrylic layer to reduce its exposure to the surrounding environment and moisture.[15] This nonpolar coating is considered a ‘‘sacrificial layer’’ able to protect the acrylic one underneath, avoiding its degradation.

Wax coverings tend to degrade over time, making the removal of this layer necessary.[1,16] They tend to embed pollutants and dust due to their low melting point (between 39–65 1C depending on the type of wax), also showing chromatic and morphological alterations.[17] Indeed, even though wax-based coatings are commonly used to protect outdoor sculptures from degradation and are still considered more beneficial maintenance products for outdoor sculptures than most other coatings, their barrier properties are affected by defects in their structure – namely, where the layer is not consistent – and their vulnerability to chemical alterations induced by weathering.[3,6,18,19] These factors are the main causes of their short lifetime – 2 to 5 years for microcrystalline waxes, which tend to exfoliate and become powdery, thus also altering the aesthetic appearance of the artwork. Indeed, the tendency to deteriorate requires constant maintenance, which means periodic cleaning treatments.[4] For this kind of intervention, hazardous solvents for both the environment and human health are still widely used. Highly flammable and toxic solvents, namely aliphatic and aromatic hydrocarbons, such as Petroleum Ether and White Spirit, are being used due to their physical-chemical properties, even though they are known to cause adverse effects to humans by both inhalation and dermal adsorption.[10,20–24] Their medium-high volatility, low cost, transparency, and purity make them ideal for these applications, while more sustainable low-polar cleaning systems capable to combine the need for the preservation of the artwork’s integrity with a greener approach are still lacking.

The role of green chemistry in improving sustainability in the Cultural Heritage conservation field is increasingly growing.[25,26] The general aim is to replace traditional hazardous methods and products that are still widely used in the field, especially for the removal of aged coatings from artistic surfaces, thus ensuring the safety of both the artworks and the operators.[27–32] Even though a low-impact and effective approach to cleaning treatments is now recognized as an urgent need, novel environmentally friendly solutions and protocols are yet to be investigated. In this framework, Deep Eutectic Solvents (DESs) can be considered promising alternatives to conventional organic solvents due to their excellent physical–chemical properties – *i.e.*, low volatility, high dissolution power, biodegradability, and low toxicity – together with their easy synthesis, accessibility of their natural compounds, low cost, and recyclability.[33] They are mixtures of two or more solid components leading to a strong depression of the melting point when compared to their individual counterparts, due to the presence of nonsymmetric ions with low lattice energy.[34] They form eutectic mixtures of a hydrogen-bonding acceptor (HBA) and a hydrogen-bonding donor (HBD) able to self-associate *via* hydrogen bonds and van der Waals interactions, inducing the charge delocalization responsible for the decrease in the melting point.[35] Hydrophilic DESs can be described by the general formula:

$$\mathrm{Cat}^{+}\,\mathrm{X}^{-}z\mathrm{Y} \qquad (1)$$

where Cat^{+} represents any ammonium, phosphonium, or sulphonium cation, X is a Lewis base – generally a halide anion – acting as a counter ion, and Y is a Lewis or Brønsted acid of which z molecules interact with the X^{-} anion.[34] The properties of the final DES are classified based on the nature of the complexing agent and can be adjusted through the selection of the individual components based on their chemical structure and molar ratio.[36,37] The discovery of these non-toxic formulations generated a breakthrough in the world of green chemistry. As eco-friendly solvents, DESs are being used in many areas of science and technology due to their excellent physicochemical properties, such as low volatility and low toxicity. However, although few attempts have been made so far to use hydrophilic DESs in the Cultural Heritage field, hydrophobic DESs have not been yet investigated.[38–41] Hydrophobic DESs can be obtained from a mixture of two components (component A and component B), a hydrogen-bonding acceptor (HBA) and a hydrogen-bonding donor (HBD), generally having no charge. Therefore, they are neutral compounds:[35,42]

[Component 1(HBA)-component 2 (HBD)] (2)

They represent promising solutions, since other potentially suitable compounds that could hypothetically replace aliphatic hydrocarbons come in solid form at room temperature and cannot be used as liquid solvents. Indeed, most of the potential alternatives to toxic solvents consist of molecules having long alkyl chains. As the length of the chain increases, the polarity of the substance decreases, while its melting point increases, making the substance solid at room temperature. The substance's physical state at standard temperatures does not allow for its utilization as a solvent in the liquid form as it needs to be. In this framework, DESs' feature to deeply reduce the melting point value can overcome this limitation and turn low-polar solid substances with medium-long alkyl chains into liquid solvents, while exploiting the solubility properties of their constituents.

The potential of hydrophobic DESs not only relies on the possibility of exploiting the properties of compounds that cannot be used as solvents at room temperature due to their physical state but also on their biocompatibility and low toxicity. These two features are of the outermost importance in the conservation of Cultural Heritage since the operators working on the removal of non-polar coatings are often subjected to inhalation of toxic solvents vapors – *i.e.*, petroleum derivatives – which may cause pathological diseases with long-term exposure.[43] For this reason, we exploited and herein propose for the first time the potential of hydrophobic DESs as new eco-friendly solvents for the removal of low-polar coatings – *i.e.*, waxes – from artistic surfaces, aiming at replacing more hazardous traditional solvents commonly used in the Cultural Heritage sector.

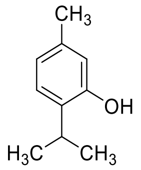
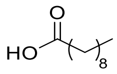
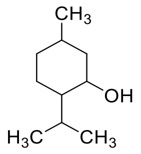
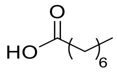
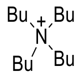
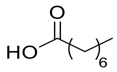
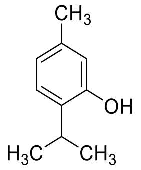
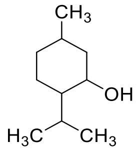
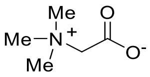
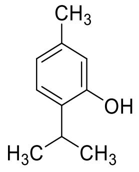
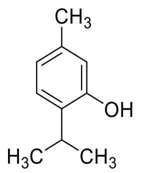
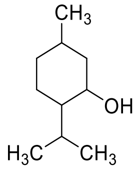
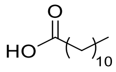
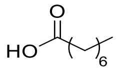
2. Experimental

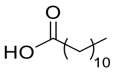
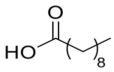
2.1 Materials and methods

Materials. Beeswax and two microcrystalline waxes that are commonly present as protective coatings on stone and metal sculptures were used. Beeswax and Renaissance® microcrystalline wax polish were purchased from Roma Restauro srl. R21 dispersion of microcrystalline wax in turpentine essence (10% w/v) was purchased from AN.T.A.RES srl. Ligroin 100– 140 1C (CAS: 8032-32-4) was provided by

I.M.A.R. Italia. Rhodamine 6G (CAS: 989-38-8) was purchased from Sigma-Aldrich®, while dearomatized White Spirit was provided by Antichità Belsito s.r.l. The eight Deep Eutectic Solvents (DES 1 – DES 8) presented in this paper and reported in Table 1 were synthesized

Table 1 Composition of the tested DESs

DES code	Component 1	Component 2	Molar Ratio	Melting point [°C]
DES 1	 Thymol	 Decanoic Acid	1: 1[44]	11.5[50]
DES 2	 L-(-)-Menthol	 Octanoic Acid	1: 1[45]	—
DES 3	 Tetrabutylammonium	 Octanoic Acid	1: 1[46]	—
DES 4	 Thymol	 L-(-)-Menthol	1: 2[46]	— 4.5[51]
DES 5	 Betaine	 Thymol	1: 3[48]	— 15[48]
DES 6	 Thymol	 L-(-)-Menthol	1: 1[47]	—
DES 7	 Dodecanoic Acid	 Octanoic Acid	1: 3[49]	—

DES 8	 Dodecanoic Acid	 Decanoic Acid	1: 2[49]	—

by the Laboratory of Industrial and Synthetic Organic Chemistry (LISOC) within the Department of Chemistry and Chemical Technologies at the University of Calabria. For the synthesis of DESs, Thymol, L-(—)-menthol, tetrabutylammonium bromide, betaine, octanoic acid, decanoic acid, and dodecanoic acid were purchased from Sigma Aldrich (Merck KGaA, Darm-stadt, Germany). Eventually, the only precursor that comes in liquid form at room temperature, namely octanoic acid, was used for the testing as well. With the only exception of DES 3, all the tested solvents consist of compounds of natural origin. Hence, they can be defined as NADES (Natural Deep Eutectic Solvents).

Hydrophobic DESs preparation. Hydrophobic DESs were synthesized by mixing component 1 and component 2 at the proper molar ratio – as shown in Table 1 – and heating them to a temperature between 80 1C and 100 1C until homogeneous liquids were formed in times ranging from 1 to 3 hours.[44–49]

Solubility tests. Solubility tests were performed by mixing 2 parts of wax and 5 parts of solvent (w/v) in vials. Along with the 8 DESs, one precursor – *i.e.*, octanoic acid – and reference solvents were tested for each wax, namely White Spirit for the beeswax and Ligroin 100–140 1C for the two microcrystalline waxes.

The vials were placed in a Vevor® digital ultrasonic cleaner (120 W, 50 Hz) to accelerate the solubilization process for 24 hours. The degree of solubilization was evaluated through turbidity measurements by filtering the mixture to remove solid residues. The analysis was performed using a Haze 3001 Turbidity Meter.

Contact angle measurements. Contact angle measurements were performed with the LAUDA Surface Analyzer LSA60 to assess the waxes' wettability using the tested formulations, thus evaluating the surface–liquid interactions. The eight DESs were examined, as well as reference solvents – *i.e.*, White Spirit and Ligroin 100–140 1C – and deionized H_2O. The analysis was carried out by bringing down a drop of each solvent on microscope glass slides covered with 0.5 g of each wax. During the preparation of the mockups, the R21 dispersion was simply applied on the slides and let harden, while Beeswax and Renaissance® were slightly heated up to 50 1C to make them melt and form a flat and uniform surface. For each solvent–wax combination, three measurements were performed to reduce the uncertainty of the analysis. The results are presented as mean values.

Cleaning tests. Laboratory specimens were prepared by applying 0.5 g of each wax, both pure and mixed with the fluorescence marker Rhodamine 6G, on microscope glass slides to evaluate the solvents' solubility on an inert substrate. The waxes were loaded with Rhodamine 6G to allow for the evaluation of the effective removal of the wax layer in the treated areas through spectrocolorimetric analysis and multi-spectral imaging. Beeswax was heated up to 50 1C, while Renaissance® up to 40 1C to facilitate their application. Rhodamine 6G was added after the heating process. Once the solid state was reached, specimens were soaked in ethanol/water solution (20 : 70) for 3 hours to remove the excess marker, thus avoiding further extraction of the sole Rhodamine 6G during the cleaning tests. Swab cleaning tests were carried out simulating the operational mode commonly used by restorers: each solvent – also comprising reference solvents (*i.e.*, White Spirit for beeswax and Ligroin 100–140 1C for microcrystalline waxes) – was left on the layers of wax in the same amount (1 mL) for increasing contact times – *i.e.*, 1 minute, 2 minutes, and 3 minutes – and then the solvent's residues were removed from the surface using cotton swabs wounded on a thin stick. Tests were summarized in the Results section by assigning an average value to each wax–solvent combination. During the treatment, mechanical rubbing action was minimized to limit its contribution to the removal of the layer. Deionized water was also used as a reference solvent, as it should not have any kind of interaction with wax. Spectrocolorimetric analyses were performed before and after the cleaning treatment using a portable spectrophotometer Y3060 3nh, equipped with a D65 illuminant and an 8 mm size aperture. The analysis was carried out in the SCI mode (Specular Component Included) and by measuring the spectra between 400 and 700 nm. The acquisitions were performed three times on each sample to reduce the uncertainty of the analysis. Data were then analyzed through the CIELab color system. The variation of the parameters L^*, a^* and b^* was calculated by evaluating the Euclidean distance between the mean of the values acquired on the areas after the treatment and the ones collected on the blank sample. ΔL^*, Δa^*, and Δb^* were summarized in the total color difference ΔE, given by the following equation:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

For each sample, ΔE uncertainty was estimated through the calculation of the standard deviation of the ΔE_s computed for each triad of measurements. Smaller values indicate greater proximity of the treated area to the sample without the wax coating, thus demonstrating the higher effectiveness of the solvent. Higher values mean that the wax layer is still present to different degrees, hence proving the less interaction between the solvent and the wax.

Multispectral imaging was performed using UV (365 nm) and visible (VIS) light sources before and after the cleaning tests to evaluate the solvents' effectiveness. The analysis was carried out using the Madatec multispectral system, which consists of a full-spectrum Samsung NX500 Digital Camera (28.2 MP BSI CMOS) and Madatec spotlights with 365 nm (UV) wavelength. Images of the induced fluorescence were taken using the HOYA UV-IR filter cut 52 and the Yellow 495. 52 mm F-PRO MRC 022 filter to reduce the component of the UV spotlight, thus better highlighting possible fluorescence effects.[52]

Fourier Transform Infrared (FT-IR) spectroscopy in the Attenuated Total Reflectance (ATR) mode was performed on the swabs used for the cleaning tests as well to further confirm the presence of the wax–solvent mixture on them, hence assessing the actual interaction between the solvent and the solute. The IR spectra were collected using the Nicolet Summit FTIR spectrometer equipped with the Everestt Diamond ATR accessory. A total of 32 scans were performed on each sample with an instrumental resolution of 8 cm^{-1}.

3. Results

Solubility tests

Fig. 1 shows the dissolution degree of the waxes in the tested solvents. Total dissolution was not achieved with any of the tested DESs, including reference solvents that are commonly used for the removal of wax layers from works of art, namely Ligroin 100–140 1C for the microcrystalline waxes and White Spirit for the beeswax. Indeed, the interaction between the waxes and the solvents resulted in turbid colloidal dispersions

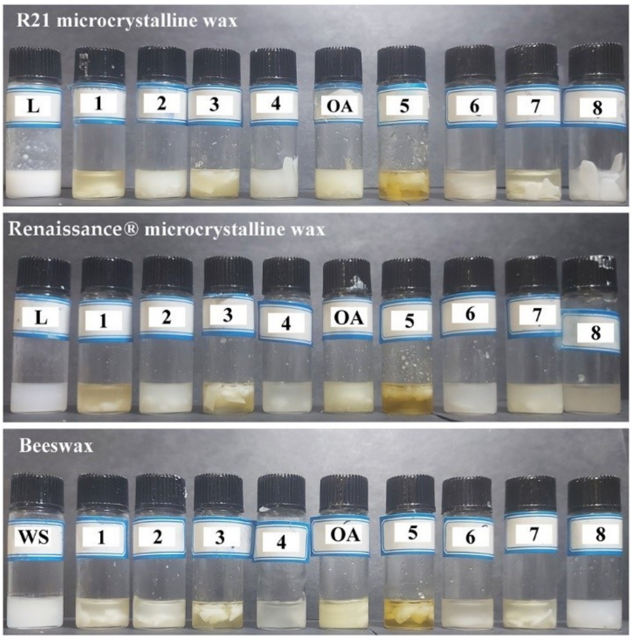


Fig. 1 Solubility tests, from the top to the bottom: R21 microcrystalline wax, Renaissance® wax, and beeswax mixed with the tested solvents in vials. L: ligroin 100–140 1C; octanoic acid: OA. DESs are labelled with consecutive numbering. Fatty acids-based DESs: 1, 2, 3, 7, 8; thymol-based DESs: 4, 5, 6.

instead of solutions. This effect is explained by the fact that the waxes form opaque colloidal systems in nonpolar organic solvents, by having particles with a size of few nanometers to 1 mm range.[53,54] Due to the formation of colloidal dispersions, turbidity measurements were used to assess the degree of solubilization of the waxes in each solvent. High Nephelometric Turbidity Unit (NTU) values imply significant cloudiness of the mixture, meaning the formation of intermolecular interactions among the solvents and the waxes with the formation of the suspension. Conversely, low NTU values highlight a lack of interaction between the wax and the solvent. Fig. 2 illustrates the NTU values for each solvent–wax combination normalized to the reference solvents, *i.e.*, Ligroin and White Spirit. The degree of dispersion of the microcrystalline wax R21 in each solvent can be defined as follows: L > DES 2 > DES 4 > O.A. > DES 6 > DES 3 > DES 1 > DES 8 > DES 5 > DES 7. As to the Renaissance® wax polish, turbidity measurements showed that: DES 2 > O.A. > DES 7 > L > DES 6 > DES 8 > DES 4 >

DES 1 > DES 3 > DES 5. Fewer Deep Eutectic Solvents had the same dispersive effect on the beeswax, probably due to the presence of more polar groups in the beeswax chemical composition (complex wax esters, linear wax monoesters and hydroxy monoesters, free fatty acids, and free fatty alcohols).[55] The turbidity of the beeswax–solvent mixtures can be summarized as follows: WS > DES 8 > O.A. > DES 2 > DES 6 > DES 1 > DES 7 > DES 4 > DES 3 > DES 5.

Contact angle measurements

Contact angle measurements demonstrated the tendency of each solvent to spread over the surface or be repelled by it, thus defining the wettability of the three waxes by the tested DESs and references (Fig. 3). The poor wettability of the substrate by water is evidenced by the high contact-angle values, ranging from 91.4° ± 1.5 to 120° ± 0.3. On the other hand, the hydrophobicity of the reference solvents and DESs caused a high reduction of the contact angle values, highlighting the overall wettability of the three waxes by these formulations. As to the microcrystalline dispersion R21, the solvents showing the lower contact angle values, meaning the higher wettability, are as follows: WS, DES 4, DES 6, DES 2, and L, while higher values were acquired for DES 8, O.A., DES 7, DES 1, DES 3, DES 5. Results obtained from the analysis of Renaissance® wax showed similar results, even though

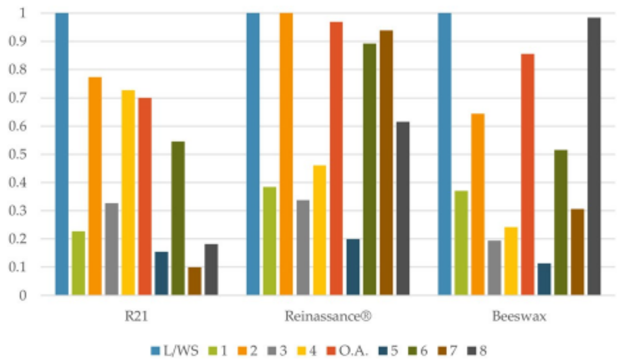


Fig. 2 Normalized values derived from turbidity measurements of solvent–wax mixtures. L: ligroin 100–140 1C (used as a reference for R21 and Renaissance®); WS: White Spirit (used as a reference for beeswax); octanoic acid: OA. DESs are labelled with consecutive numbering. Fatty acids-based DESs: 1, 2, 3, 7, 8; thymol-based DESs: 4, 5, 6.

outlining the general lower wettability by all the hydrophobic solvents, except for White Spirit. Indeed, only WS and DES 2 provided values lower than 20° – respectively 2.2° ± 1.3 and 19.9° ± 1.6. The results related to the other formulations can be summarized as follows (from the lower to the higher values): L, O.A., DES 8 = DES 6, DES 1, DES 7, DES 4, DES 3, DES 5.

Beeswax exhibited a general lower wettability by the tested solvents, whose contact-angle values are in the following order (from the lower to the higher one): WS, DES 2, DES 8, DES 1 = DES 7 = O.A., DES 6, DES 4, L, DES 3, DES 5.

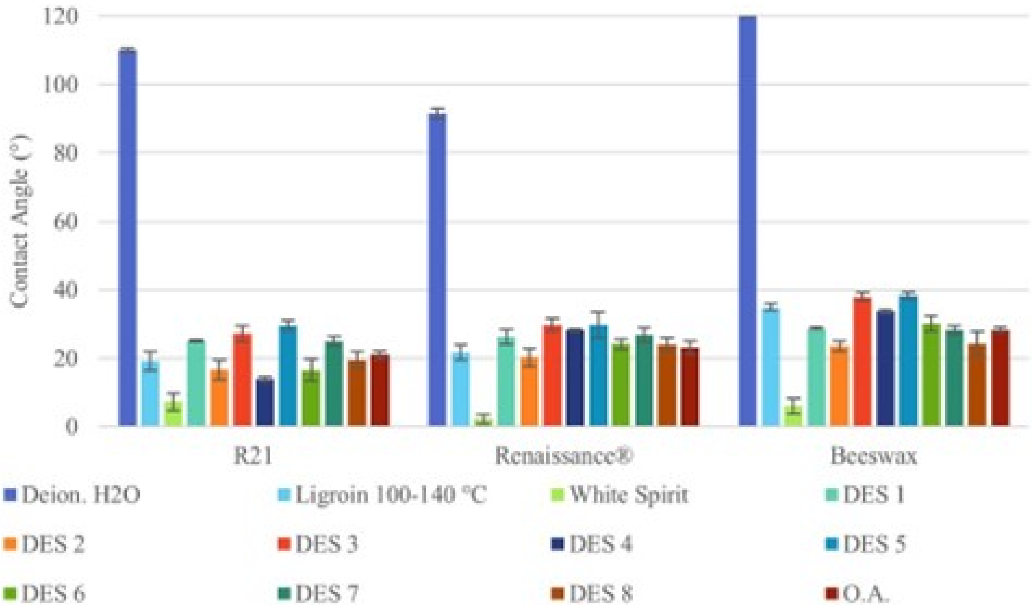


Fig. 3 Contact angle values: mean resulting from three measurements and standard deviation.

Cleaning tests

Spectrocolorimetric analysis. Table 2 reports the results of the spectrocolorimetric measurements, which enabled the definition of the DESs cleaning performance on an inert substrate ‑ i.e., glass slides ‑ hence assessing the effective removal of the coating by each solvent. The chromatic variation (ΔE) was calculated by comparing the chromatic values obtained after the cleaning tests with the ones of the microscope glass' (blank sample), thus describing the ability of the tested solvents in removing the wax. High DE values denote the presence of wax on the sample’s surface, while low ones indicate the higher effectiveness of the solvent.

For R21, the solvents’efficacy based on spectrocolorimetric analysis can be defined as follows: L > DES 2 > DES 6 > DES 4 > DES 1 > DES 8 > DES 7 > O.A. > DES 3 > DES 5 > H_2O. As to the Renaissance® wax polish, the results can be summarized as L > DES 2 > DES 4 > DES 7 > O.A. > DES 6 > DES 8 > DES 1 > DES 5 > DES 3 > H_2O. Finally, beeswax’s ΔE values can be explained as WS > DES 2 > DES 8 > DES 1 > DES 7 > DES 4 > DES 6 > DES 5 > DES 3 > H_2O. The observations made during the removal of the wax layer, coupled with the results of the spectrocolorimetric measurements, enabled the definition of the cleaning performance on an inert substrate - i.e., glass slides - hence assessing the effective removal of the coating by each solvent. As a general remark, longer contact times (i.e., 3 minutes) allow for more efficient removal of the layer. Also, more hydrophobic DESs seem to interact with beeswax, while lesser with the microcrystalline wax R21. Indeed, beeswax is particularly responsive to DES 2 and 8, but also to DES 1, resulting in the following descending ranking: WS = DES 2 = DES 8, DES 1, DES 4 = O.A. = DES 7, DES 6, DES 3 = DES 5. The results obtained from the cleaning tests on Renaissance® can be summarized in descending order as L = DES 2, DES 4, O.A. = DES 7, DES 1 = DES 8, DES 3 = DES 5 = DES 6 while, as to R21, only DES 2 almost equals the result of the reference solvent (Ligroin). The other solvents appear to be able only to partially remove the wax layer, in the following descending sequence: L, DES 2, DES 1 = DES 4 = DES 6 = DES 8, DES 3 = O.A. = DES 7, DES 5. The

Table 2 Spectrocolorimetric parameter Δ*E* for each solvent–solute combination, resulting from the mean of three measurements. The standard deviation computed on ΔE_s is 0.47 < SD < 7.95

	ΔE_s values		
Solvent	R21	Renaissance^{s}	Beeswax
Deionized water	28.64	17.41	7.87
DES 1	13.66	16.02	3.06
DES 2	8.04	0.76	2.38
DES 3	24.21	16.77	7.01
DES 4	13.34	4.58	5.56
DES 5	27.01	16.76	7.00

DES 6	12.99	15.55	6.19
DES 7	22.86	9.96	5.34
DES 8	14.03	15.83	2.54
Ligroin/white spirit	4.44	0.63	2.33
Octanoic acid	23.67	10.46	5.69

Table 3 Average rating assigned during the cleaning tests. Legend: – stands for "no" removal", while ***** indicates complete removal of the wax layer

Solvent	R21	Renaissances	Beeswax
Deionized water	-	-	-
Ligroin or white spirit	*****	*****	*****
DES 1	***	**	****
DES 2	****	*****	*****
DES 3	**	*	*
DES 4	***	****	***
Octanoic acid	**	***	***
DES 5	*	*	*
DES 6	***	*	**
DES 7	**	***	***
DES 8	***	**	*****

results reported in Table 3 summarize the outcome of the cleaning tests, expressed as an average rating based on the observations made during the removal considering the three different contact times.

Multispectral imaging. Multispectral imaging allowed showing the solvents' effectiveness in removing the waxes, supporting the observations made during the cleaning tests. The results obtained using three DESs – namely, DESs 1, 2, and 6 – are reported in Table 4 in both visible (VIS) and UV light, as an illustration of the different degrees of cleaning effectiveness defined in Tables 2 and 3. Furthermore, the differences given by the increasing contact times are visible in the pictures shown in Table 4. Thus, the results suggest the need for longer application times that are made possible by the negligible evaporation rate of the hydrophobic DESs, which also reflects their low impact on the operator, which is safeguarded from their inhalation.

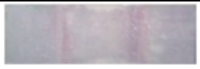
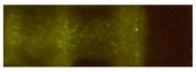
FT-IR ATR. The presence of the wax–solvent mixture on the swabs used for the cleaning treatment was evaluated through FT-IR ATR. The analysis was performed on the swabs used for the cleaning tests to assess the presence of a mixture of the DESs and the waxes on all of them, thus confirming the actual formation of the colloidal dispersion during the cleaning treatment. The spectra were interpreted considering the peaks related to the presence of cellulose-based swabs, that were prior acquired as blank determination.

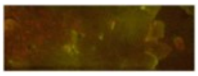
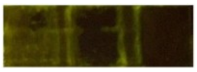
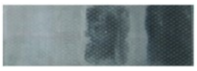
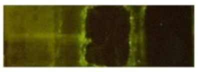
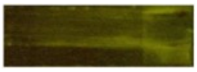
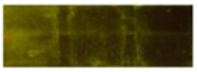
Indeed, the absorption band at 3391 cm^{-1} is assigned to hydroxyl groups stretching.[56] Bands at 2906 cm^{-1} and 1373 cm^{-1} are assigned to stretching and deformation vibrations of the C–H group that can be either related to the glucose unit of cellulose, the DESs, or the waxes (Fig. 4). The absorption band at 898 cm^{-1} is characteristic of b-glycosidic linkage between glucose units.

The signal at 1061 cm^{-1} is assigned to the –C–O– group of DESs' precursors and secondary alcohols or ethers functions existing in the cellulose chain backbone. Nevertheless, multiple peaks can be specifically attributed to the DESs or the wax, providing information about the dispersion of the substance.

As an example, the spectra acquired on the swabs used for the treatment of each type of wax with DES 1 are shown in Fig. 4‑6.

Table 4 Multispectral imaging in VIS and UV (365 nm) lights showing the effects of DES 1, DES 2, and DES 6 on the three waxes based on the increasing contact times (10, 20, and 30). SOL: solvent; RAD: radiation; R21: microcrystalline wax R21; RE: microcrystalline wax Renaissance®; BE: beeswax

			Before (VIS)			After (VIS)		
Wax	Sol	RAD	1'	2'	3'	1'	2'	3'
R21	DES 1	VIS						
		UV						
	DES 2	VIS						
		UV						

	DES 6	VIS		
		UV		
RE	DES 1	VIS		
		UV		
	DES 2	VIS		
		UV		
	DES 6	VIS		
		UV		
BE	DES 1	VIS		
		UV		
	DES 2	VIS		
		UV		
	DES 6	VIS		
		UV		

As to the microcrystalline wax R21, the characteristic peaks at 725 cm^{-1} (C–H rocking of alkenes), 2850, and 2920 cm^{-1} (C–H stretching of alkenes) are visible in all the acquisitions,

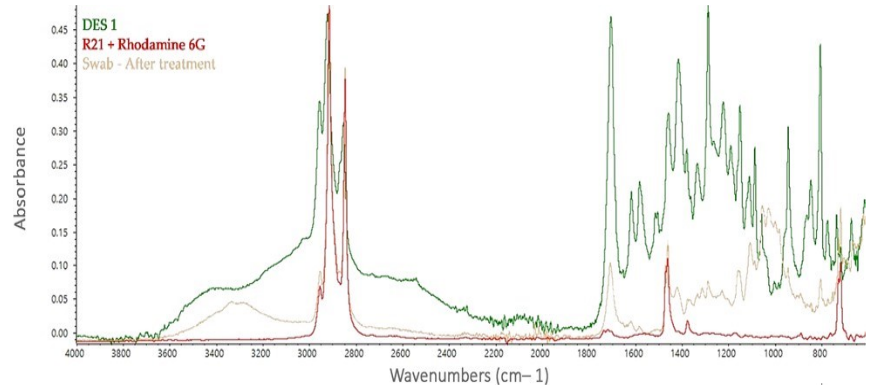


Fig. 4 FT-IR ATR spectra comparing DES 1, R21 mixed with Rhodamine 6G, and the swab used for the cleaning treatment.

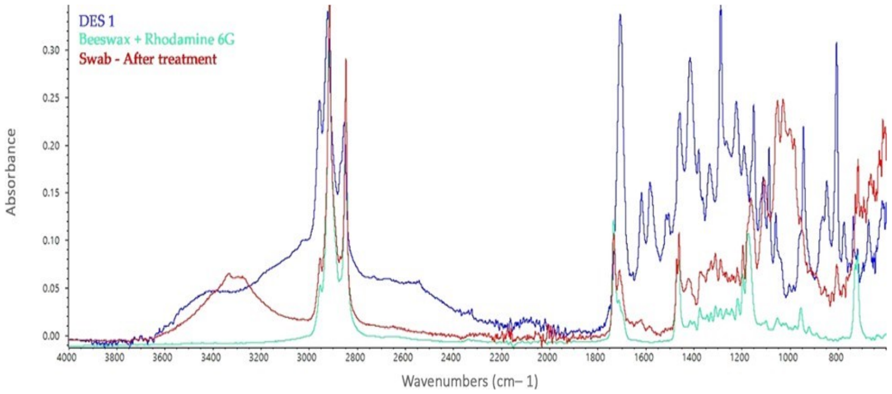


Fig. 6 FT-IR ATR spectra comparing DES 1, beeswax mixed with Rhoda- mine 6G, and the swab used for the cleaning treatment.

except for deionized water (Fig. 4).[57] At the same time, the peaks at 1720 cm^{-1} of C=O stretching of carboxylic acids, 1575 and 1620 cm^{-1} of C=C stretching of cyclic and conjugated alkenes, 1220 and 1290 cm^{-1} of C–O stretching, 815 cm^{-1} of CQC alkene bending can be associated with the solvent.

Due to the nature of the two microcrystalline waxes, the Renaissance® wax shows the same characteristic peaks as the R21 (Fig. 5). Hence, the same considerations can be made, also due the presence of the same bands related to the three DESs that can be seen in the previous spectra.

As to beeswax, the characteristic peaks at 2850 and 2920 cm^{-1} (C–H stretching of alkenes), 1740 cm^{-1} (C=O stretching of esters), 1460 cm^{-1} (C–H bending of alkanes), 1150 cm^{-1} (C–O stretching), 960 cm^{-1} and 725 cm^{-1} (C=C bending of alkenes) can be seen in the spectrum acquired on the swab after the treatment (Fig. 6) (Dubey, Sharma, & Kumar, 2017). Additionally, the peaks at 1720 cm^{-1} of C=O stretching of carboxylic acids, 1425 cm^{-1} of O–H bending, and 1150 cm^{-1} of C–O stretching are related to the solvent.

4. Discussion

The degree of dispersion of the waxes in the tested solvents was examined. The results showed that DES 2 and DES 4 produce a higher degree of dispersion on the microcrystalline wax R21, while DES 2, octanoic acid, and DES 7 showed the same good results in the dispersion of the microcrystalline wax Renaissance®. Also, DES 8, octanoic acid, and DES 2 effectively interacted with beeswax. However, it is worth mentioning that the degree of dispersion hinges not only on the affinity between the solvent and the dispersed phase but also on their relative proportion. The presence of solid residues of wax could be related to the oversaturation of the dispersion, thus suggesting that complete dispersion of the wax can be obtained with a higher solvent volume. The R21-DES4, beeswax-DES2, beeswax-O.A., beeswax-DES8, and Renaissance®-DES2 mixtures were highly opaque, possibly entailing the saturation of the dispersion and, in

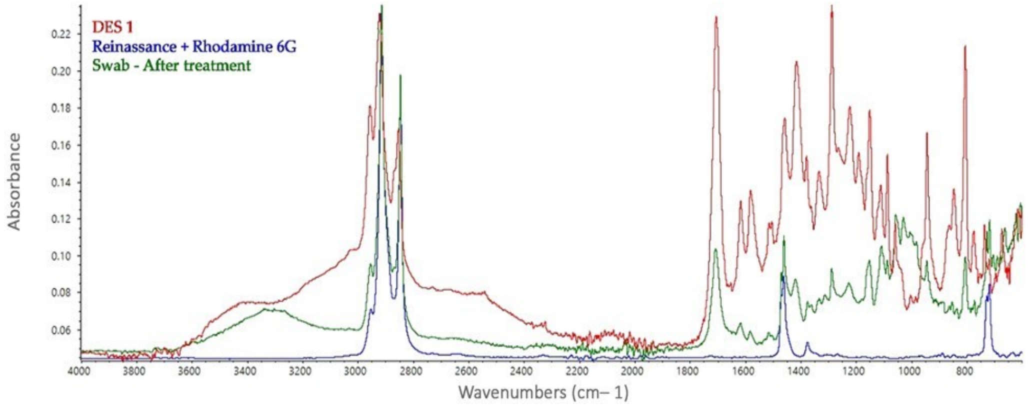


Fig. 5 FT-IR ATR spectra comparing DES 1, Renaissance® mixed with Rhodamine 6G, and the swab used for the cleaning treatment.

the case of the R21-DES4, beeswax-DES2, and beeswax-O.A., leading to the formation of a semi-solid paste.

Contact angle measurements highlighted the general tendency of the DESs to spread onto the waxes' layers as opposed to deionized water, due to the higher affinity between the DESs and the solute having high hydrophobicity. Cleaning tests enabled the direct evaluation of the solvents' effectiveness by using the operating procedure commonly employed by restorers – *i.e.*, the swab-cleaning method – thus providing valuable information on the DESs' actual potential for their use as new sustainable cleaning systems for the removal of non-polar substances from Cultural Heritage materials. The conclusions resulting from the cleaning tests, multispectral imaging, and spectrocolorimetry show that DES 2 almost equals the efficacy of Ligroin in removing the R21 microcrystalline wax, while DES 2 and DES 4 have a greater effect on the cleaning of the Renaissance^s wax polish. In addition, DES 2, DES 8, and DES 1 were able to remove the beeswax layer successfully. Also, all the proposed formulations formed a semi-solid paste with the wax, allowing for easy removal of the solvent–solute mixture from the specimens' surface.

FT-IR ATR analyses confirmed the actual interaction between the solvent and the solute during the cleaning treatment, thus disproving the hypothesis that the removal of the layer may have been due to mechanical action. Indeed, the presence of both solvent and wax residues on the swabs used for the cleaning treatment showed that the agglomerated particles of the wax are effectively separated from each other through the action of the solvent.

Based on the whole study, it is possible to conclude that the combination L-(—)-menthol–octanoic acid 1 : 1 (DES 2) shows a higher affinity with the three types of wax. Besides, promising results are also given by the application of DES 2 on the microcrystalline wax Renaissance^s and by the utilization of dodecanoic acid–decanoic acid 1 : 2 (DES 8), followed by DES 2, and thymol–Decanoic acid 1 : 1 (DES 1) for the removal of beeswax. The different solvent–solute interactions observed in the experimentation can be related to the different compositions of the three waxes. Indeed, microcrystalline waxes are produced by de-oiling petrolatum, as part of the petroleum refining process. While paraffin wax contains mostly unbranched alkanes, microcrystalline wax is composed of a higher percentage of isoparaffinic (branched) hydrocarbons and naphthenic hydrocarbons.[56] Indeed, the presence of cyclic hydrocarbons in their composition explains the better dissolution of the micro-crystalline wax R21 in DESs based on L-(—)-menthol. Their respective structures interact due to the formation of weak intermolecular forces involving the hydrophobic portions of the molecules – *i.e.*, cyclic hydrocarbons. This phenomenon becomes particularly evident when DES 4 and DES 6 are compared. While DES 6 consists of equal proportions of L-(—)-menthol and thymol – containing an aromatic structure instead – DES 4 has twice as much L-(—)-menthol, thus causing greater dissolution of the solute.

Even though the Renaissance® wax polish is a microcrystalline wax as well, it does not contain naphthenic hydrocarbons. According to the information provided by producers, it is a mixture of waxes containing polyethylene wax – *i.e.*, ultra-low molecular weight polyethylene based on ethylene monomer chains – which makes it more stable compared to other microcrystalline waxes intended for conservation.[58]

This difference in the composition between the two microcrystalline waxes may be the cause of their different interaction with the tested solvents. Octanoic acid seems to have greater compatibility with the Renaissance® wax, probably due to the chain length of low-molecular weight-based waxes. Indeed, low molecular-weight waxes might have C8–C9 alkyl chains which could better interact with the alkyl chains of octanoic acid.

Even though differences have been pointed out between the two microcrystalline waxes, the intermolecular forces driving their interaction with the proposed solvents presumably consist of London dispersion forces involving the linear alkyl chains of the solvents – *i.e.*, carboxylic acids – the branched hydrocarbons of the waxes, and the cyclic structures of both L-(—)-menthol and R21 microcrystalline wax.

Eventually, beeswax is a complex mixture of hydrocarbons (12–16%) with a predominant chain length of C27–C33, free fatty acids (12–14%), with a chain length of C24–C32, linear wax monoesters and hydroxy monoesters (35–45%) with chain lengths generally of C40–C48, complex wax esters (15–27%), and exogenous substances.[53] The presence of long alkyl chains, –OH, $–COOR'$, and –COOH groups in beeswax' structure explains the broad range of solubility of beeswax in low- and medium-polar solvents.[56] Also, the higher interaction between beeswax and DES 8 can be related to the presence of fatty acids having –COOH groups, similar to beeswax, and the longer alkyl chains among the tested acids. As to carboxylic acids containing DESs, the interaction between the solvents and beeswax can be mainly ascribed to the formation of hydrogen bonds between the polar moieties of the solvent and the beeswax. The carbonyl group –C=O of carboxylic acids can form hydrogen bonds with both the hydroxyl groups –O–H of fatty acids and wax hydroxy monoesters contained in the beeswax. Likewise, the hydroxyl group of the solvent can form hydrogen bonds with the carbonyl group of wax's fatty acids, monoesters, and hydroxy monoesters. London dispersion forces play a significant role as well, by involving the hydrophobic moieties of the solvent and the solute. These interactions tend to increase with increasing the chain length, due to the higher number of electrons able to generate more instantaneous dipoles. Indeed, the reduction of the alkyl chain's length – as with octanoic acid-based and L-(—)-menthol or thymol-based DESs – the action on beeswax decreases, even though the hydroxyl groups should partially interact with the ones present in the wax.

5. Conclusions

The experimental process provided valuable results proving hydrophobic DESs' potential of being used as sustainable solvents for cleaning treatments on Cultural Heritage materials. Hydrophobic Natural Deep Eutectic Solvents proved to be potentially suitable alternatives to more toxic organic solvents that are currently popular in the conservation of Cultural Heritage sector. Indeed, NADESs have low toxicity for the operator, as they consist of natural and biocompatible sub-stances and have low volatility. This latter aspect has a twofold implication: a reduced amount of solvent is needed, since it does not evaporate and continues to interact with the coating to be removed as long as required, making the treatment fully sustainable; the operator does not inhale solvent vapors, thus avoiding health risks. Also, the low evaporation rate allows for the recovery of the DESs, which can be extracted from the solvent–solute mixture and reused. Several recycling methods are currently being studied for both hydrophilic and hydrophobic deep eutectic solvents – *i.e.*, adsorption with activated carbon, back extraction, anti-solvent addition, crystallization, liquid–liquid extraction, solid–liquid extraction, short-path distillation, supercritical fluid extraction, membrane-based processes, and separation due to density and viscosity differences.[59–61] In addition, DESs have physical properties that make them suitable for use in cleaning operations, such as transparency, moderate viscosity, and effective interaction with the material to be removed.

In the present study, the potential of different NADES has been revealed. Specifically, L-(—)-menthol-based DESs–thymol/ L-(—)-menthol 1 : 2 in particular – provided positive results in combination with the R21 microcrystalline wax, presumably due to the presence of naphthenic hydrocarbons in the wax' composition. Octanoic acid-based DESs – *i.e.*, L-(—)-menthol/ cctanoic acid 1 : 1 and dodecanoic acid/octanoic acid 1 : 3 – showed higher interaction with the Renaissance® wax, probably due to the alkyl chains' length of low-molecular-weight polyethylene waxes and branched hydrocarbons that may resemble that of octanoic acid. Eventually, fatty acid-based DESs with the longer alkyl chains – *i.e.*, dodecanoic acid/ decanoic acid 1 : 2, L-(—)-menthol/octanoic acid 1 : 1 – show a greater affinity with beeswax due to the presence of the same –COOH functional groups and long alkyl chains in the structure of beeswax' constituents.

Future research will focus on the definition of the solubility parameters of hydrophobic deep eutectic solvents to determine the solubility parameters and the areas of the Teas Triangle in which they stand. The cleaning performance will be also evaluated on mockups reproducing Cultural Heritage works, such as stone and metal surfaces, examining once again the presence of solvent residues and the interaction between the hydrophobic NADESs and the substrate.

Author contributions

Chiara Biribicchi: conceptualization, methodology, data curation, writing – original draft preparation, visualization, investigation, writing – reviewing and editing, formal analysis. Andrea Macchia: conceptualization, methodology, data curation, writing – reviewing and editing, formal analysis, supervision, project administration. Gabriele Favero: validation, resources. Romina Strangis: resources. Bartolo Gabriele: resources. Raffaella Mancuso: resources, writing – original draft preparation. Mauro Francesco La Russa: project administration.

Conflicts of interest

There are no conflicts to declare.

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Chapter 3

Article: Iodocyclization of 2-Methylthiophenylacetylenes to 3-Iodobenzothiophenes and their coupling Reactions under More Sustainable Conditions

Role and contributions of the candidate**:** In this research, my main roles included preparation of DESs and active participation in the experimental section and characterization of the synthesized molecules.

This article was published in Asian Journal of Organic Chemistry, volume 11, issue 9, in 2022, pp. e202200353. Available at: https://doi.org/10.1002/ajoc.202200353. Intitled "Iodocyclization of 2-Methylthiophenylacetylenes to 3-Iodobenzothiophenes and their coupling Reactions under More Sustainable Conditions", the authors are Raffaella Mancuso,*[a] Melania Lettieri,[a] Romina Strangis,[a] Patrizio Russo,[a] Antonio Palumbo Piccionello,[b] Sara De Angelis,[a, c] and Bartolo Gabriele*[a]

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Abstract: We report the first example of iodocyclization of readily available 2-methylthiophenylacetylenes in a deep eutectic solvent (ChCl/urea 1/2, mol/mol) as recyclable and more sustainable solvent with respect to the classical VOCs employed so far. The process successfully afforded a variety of 3-iodobenzothiophenes in good to high yields starting from differently substituted substrates, with the possibility to recycle the DES several times without appreciable lowering of the product yield. The 3-iodothiophenes thus synthesized are known to be important precursors of biologically active molecules and functionalized heterocycles, and were successfully employed for performing representative Sonogashira and Suzuki cross-coupling reactions. The solvent-catalyst system could be conveniently recycled several times without any loss of activity in both coupling processes, thus further demonstrating the practical usefulness of our approach.

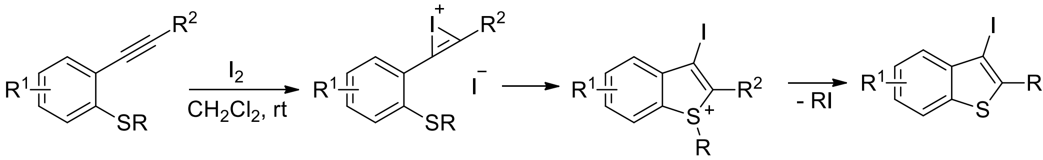
1. Introduction

3-Iodobenzothiophenes are very important benzothiophene derivatives. In fact, they are useful precursors for the formation of biologically relevant compounds[1] and as cross-coupling partners for the synthesis of functionalized benzothiophene derivatives and complex molecular architectures.[2] A simple and very convenient approach to the synthesis of 3-iodobenzothiophenes consists in the iodocyclization[3] of 2-alkylthiophenylacetylenes, as shown in Scheme 1 (R=Bn, Me). This process was first disclosed by Flynn (with R=Bn)[4] and Larock (with R=Me)[5] in 2001, and takes place through the formation of an iodonium intermediate that undergoes *anti* attack of sulfur atom of the thiomethyl group followed by sulfur demethylation by the ensuing iodide anion (Scheme 1). Since then, this approach has been further developed and elaborated by the same research groups[6,7] and others[8] under various reaction conditions. However, to the best of our knowledge, all procedures reported so far have made use of VOCs (volatile organic compounds) as solvents (including methylene chloride, 1,2-dichloroethane and ethanol). On the other hand, the replacement of VOCs with non-volatile, non-flammable, less toxic, and more sustainable solvents is becoming a more and more stringent requirement in current organic synthesis.[9] Among the most promising unconventional solvents, which may comply with these characteristics, DES (deep eutectic solvents) are now playing a major role, due to their low toxicity, very low volatility, and non-flammability. Moreover, DES can be easily and inexpensively prepared from largely available natural sources and can also be conveniently recycled.[10]

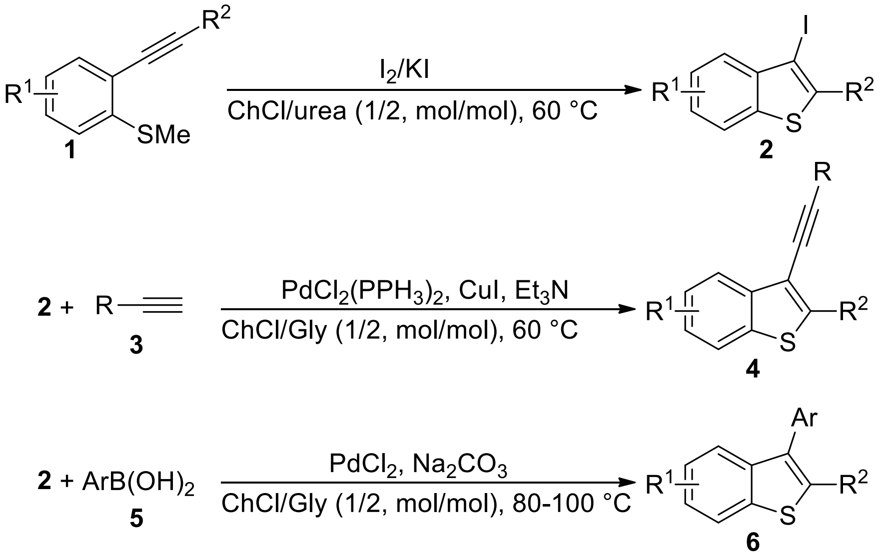
In this work, we report on the use of particularly simple and inexpensive choline chloride (ChCl)-based DES[11,12] as more sustainable and recyclable solvents for the iodocyclization of 2- methylthiophenylacetilenes **1** to 3-iodobenzothiophenes **2** in good to excellent yields. ChCl-based DES have then been successfully employed for the conduction of paradigmatic examples of Sonogashira and Suzuki couplings of **2**, with the possibility to recycle both the solvent and the catalytic systems (Scheme 2).

2. Results and Discussion

2-Methyl-4-(2-(methylthio)phenyl)but-3-yn-2-ol **1a** was chosen as model substrate for optimization experiments. The reaction of **1a** with 2 equiv. of I_2, carried out in ChCl/urea (1/2, mol/mol) as the solvent (0.15 mmol of **1a** per mL of DES) at 80 °C for 18 h, mainly led to substrate decomposition, with formation of the desired 2-(3-iodobenzo[*b*]thiophen-2-yl)propan-2-ol **2a** in only 9% yield (Table 1, entry 1). This result could be significantly improved when adding 2 equiv. of KI, which probably favors sulfur demethylation, with formation of **2a** in 68% isolated yield

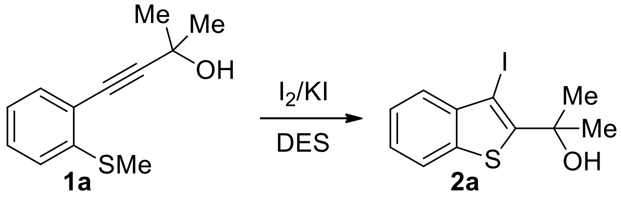


Scheme 1. Iodocyclization of 2-alkylthiophenylacetylenes to 3 iodobenzothiophenes.



Scheme 2. *This work:* Iodocyclization of 2-methylthiophenylacetylenes **1** to 3-iodobenzothiophenes **2** and subsequent Sonogashira and Suzuki couplings of **2** with terminal alkynes **3** and arylboronic acids **4**, respectively, to benzothiophenes **4** and **6**, in ChCl-based DES.

Table 1. Iodocyclization of 2-methyl-4-(2-(methylthio)phenyl)but-3-yn-2-ol **1a** in DES under different conditions.[a]



Entry	I_2 (equiv.)	KI (equiv.)	DES solvent [mol/mol]	T [°C]	Yield of **2a** [%][b]
1	2	0	ChCl/urea (1/2)	80	9
2	2	2	ChCl/urea (1/2)	80	68
3	2	5	ChCl/urea (1/2)	80	69
4	2	8	ChCl/urea (1/2)	80	67
5	2	10	ChCl/urea (1/2)	80	58
6[c]	2	10	ChCl/urea (1/2)	80	49

7	2	2	ChCl/glycerin (1/2)	80	40
8	2	2	ChCl/ethylene glycol (1/2)	80	53
9	1.5	2	ChCl/urea (1/2)	80	51
10[d]	2	2	ChCl/urea (1/2)	80	59
11	2	2	ChCl/urea (1/2)	60	81
12	1	2	ChCl/urea (1/2)	60	59

[a] Unless otherwise noted, all reactions were carried out for 18 h with a substrate concentration of 0.15 mmol per mL of DES. [b] Isolated yield based on starting **1a**. Unless otherwise noted, substrate conversion was quantitative. [c] The reaction was carried out for 15 h, and substrate conversion was 89%. [d] Substrate concentration was 0.10 mmol of **1a** per mL of DES.

under the same conditions (Table 1, entry 2). The use of a larger excess of KI did not cause a further yield increase (Table 1, entries 3–6), while the **2a** yield dropped by changing the solvent to ChCl/glycerol (1/2, mol/mol; Table 1, entry 7) or to ChCl/ethylene glycol (1/2, mol/mol; Table 1, entry 8). A lower **2a** yield was also observed when the amount of I_2 was lowered to 1.5 equiv. (Table 1, entry 9) or when conducting the process under more diluted conditions (Table 1, entry 10). On the other hand, a very good yield of **2a** (81%) was achieved under the same conditions of entry 2, when the reaction temperature was lowered to 60 °C (Table 1, entry 11). Also at 60 °C, the **2a** yield was lower when reducing the amount of I_2 (Table 1, entry 12). The optimized conditions were then applied to other differently substituted substrates. As can be seen from the results reported in Table 2, the method was of general applicability. In fact, excellent results were observed with functionalized substrates bearing a 3-hydroxyalkynyl substituent (**1a**–**g**), as shown in Table 2, entries 1–7. Substrates with the triple bond substituted with a phenethyl (**1h**; Table 2, entry 8), a simple alkyl group (**1i**; Table 2, entry 9), a sterically demanding alkyl group (**1j**; Table 2, entry 10), trimethylsilyl (**1k**; Table 2, entry 11), 1-cyclohexenyl (**1l**; Table 2, entry 12), an aryl group (**1m**–**o**; Table 2, entries 13–15), or a heteroaryl group (such as 3- thienyl, **1p**; Table 2, entry 16) also reacted well, the corresponding iodocyclization products being formed in 71–89% yields. Substrates **1q** and **1r**, bearing an electron-donating (Me) or electron-withdrawing (F) on the aromatic ring, also reacted well, with formation of the corresponding 3-iodobenzothiophenes **2q** and **2r** in 68% and 77% yields, respectively (Table 2, entries 17 and 18). The possibility to recycle the DES solvent several times, without appreciable yield loss, was also assessed for representative substrates, as shown in Table 2, entries 2, 9, 13, and 15.

We then verified the possibility to carry out paradigmatic examples of Sonogashira and Suzuki cross-couplings in a ChCl-based DES as the solvent, using the synthesized 3-iodobenzo- thiophenes **2** and different 1-alkynes **3** as coupling partners. We first tested the Sonogashira coupling of 2-(3- iodobenzo[*b*]thiophen-2-yl)propan-2-ol **2a** and 1-hexyne **3a** in the same DES solvent successfully employed for iodocyclizations [ChCl/urea (1/2, mol/mol)]. As shown in Table 3, entry, 1, the reaction led to the desired 2-(3-(hex-1-yn-1- yl)benzo[*b*]thiophen-2-yl)propan-2-ol **4aa** in 48% yield. On the other hand, by changing the solvent to ChCl/Gly (1/2, mol/mol; Gly =glycerin), the product yield improved significantly (77%, Table 3, entry 2). Accordingly, all the other experiments, leading to differently substituted 3-alkynylbenzothiophenes **4**, were carried out in this DES medium. As can be seen from the results reported in Table 3, entries 2–10, good yields of the coupling products were obtained in all the cases tested. Moreover, it has been possible to recycle the solvent-catalyst system (DES containing the palladium and copper catalysts still dissolved at the end of the process) several times without any decrease of the product yield, as exemplified in entries 6 and 9.

The results obtained in some representative Suzuki cross-couplings in ChCl/Gly (1/2, mol/mol) as the solvent are shown in Table 4. Also in this case, the process was of general applicability, and the solvent-catalyst system could be successfully recycled several times (Table 4, entries 1, 5, and 7).

3. Conclusion

In conclusion, we have shown that it is possible to perform the iodocyclization of 2-methylthiophenylacetylenes to 3-iodobenzothiophenes (particularly important precursors of biologically active molecules and functionalized heterocycles) in DES (ChCl/urea 1/2, mol/mol) as recyclable and more sustainable solvent with respect to the classical VOCs employed so far. Another simple DES (ChCl/Gly 1/2, mol/mol) has also been successfully used as solvent for performing representative Sonogashira and Suzuki cross-coupling reactions using the synthesized 3-iodothiophenes. The solvent-catalyst system has been easily recycled without any loss of activity in both coupling processes, thus further showing the practical usefulness of our approach.

4. Experimental Section

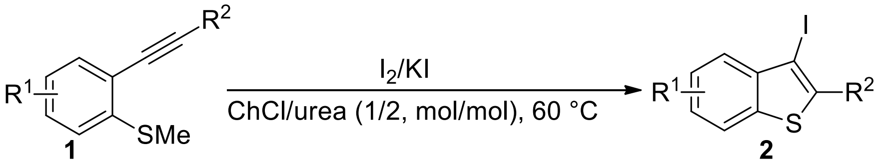
General Experimental Methods: Solvent and chemicals were reagent grade and were used without further purification. All reactions were analyzed by TLC on silica gel 60 F254 and by GLC using capillary columns with polymethylsilicone + 5% phenylsilicone as the stationary phase. Column chromatography was performed on silica gel 60 (70–230 mesh) or neutral alumina (90–170). Evaporation refers to the removal of solvent under reduced pressure. Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 25 °C on a 300 or 500 Spectrometer in $CDCl_3$ and DMSO-d_6 with Me_4Si as internal standard. Chemical shifts (δ) and coupling constants (J) are given in ppm and in Hz, respectively. IR spectra were taken with an FT-IR spectrometer. Mass spectra were obtained using a GC-MS apparatus at 70 eV ionization voltage (normal resolution) and by electrospray ionization mass spectrometry (ESI-MS) (high resolution) with a UHD accurate-mass Q-TOF spectrometer equipped with a Dual AJS ESI source working in positive mode, and were recorded in the 150–1000 m/z range. The LC-MS experimental conditions were as follows: N_2 was employed as desolvation gas at 300 °C and a flow rate of 9 L/min. The nebulizer was set to 45 psig. The Sheat gas temperature was set at 350 °C and a flow of 12 L/min. A potential of 3.5 kV was used on the capillary for positive ion mode. The fragmentor was set to 175 V.

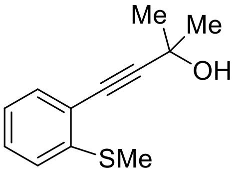
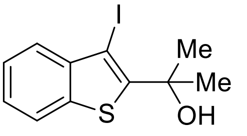
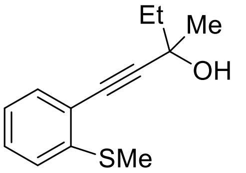
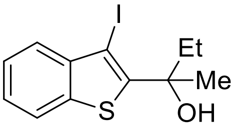
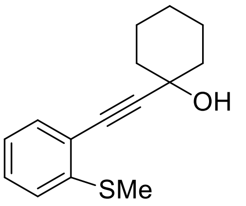
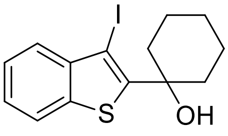
Preparation of DESs and Substrates: DESs [ChCl Gly (1 : 2 mol/mol); ChCl Urea (1 : 2 mol/mol); ChCl EG (1 : 1 mol/mol)] were prepared as we already reported.[13] Starting materials **1** were prepared as described in the Supporting Information.

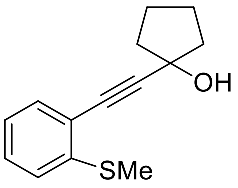
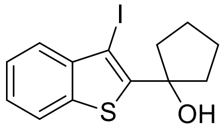
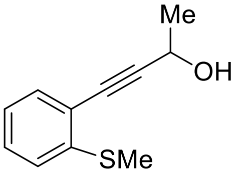
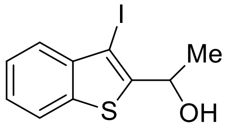
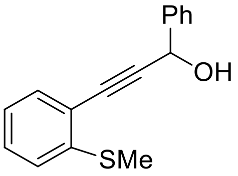
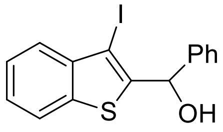
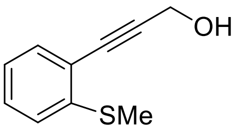
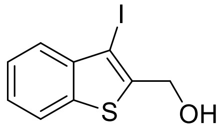
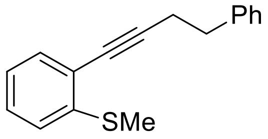
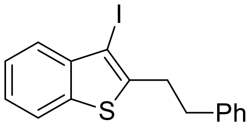
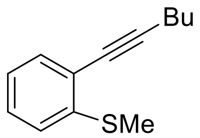
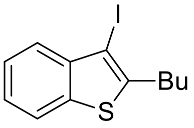
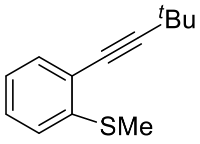
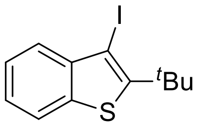
Iodocyclization procedure leading to 3-iodothiophenes 2 in DES (Table 2): To a solution of **1** (0.30 mmol) (**1a**, 62.5 mg; **1b**, 67.0 mg; **1c**, 73.5 mg; **1d**, 70.2 mg; **1e**, 58.2 mg; **1f**, 76.0 mg; **1g**, 53.7 mg; **1h**, 76.0 mg; **1i**, 61.5 mg; **1j**, 61.7 mg; **1k**, 66.2 mg; **1l**, 68.2 mg; **1m**, 67.5 mg; **1n**, 71.6 mg; **1o**, 90.4 mg; **1p**, 69.2 mg; **1q**, 71.6 mg; **1r**, 72.8 mg) in ChCl/Urea (1 : 2, mol/mol; 2.0 mL) was added I_2 (152 mg, 0.60 mmol) and KI (99.5 mg, 0.60 mmol). The mixture was allowed to stir at 60 °C for 18 h and then extracted with Et_2O (6 × 3 mL) at 60 °C. After cooling, saturated aqueous $Na_2S_2O_3$ (15 mL) was added to the collected ethereal phases, and the mixture was allowed to stir for 10 min. Phases were separated, the aqueous phase was extracted with Et_2O (3 × 15 mL), while the DES phase was used for the next recycling experiments (see below). The collected organic layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, products **2a**–**r** were purified by column chromatography on silica gel using as eluent pure hexane to 9 : 1 hexane- AcOEt (**2a**–**d**, **2g**), pure hexane to 95 : 5 hexane-Et_2O (**2f**), pure hexane to 95 : 5 hexane-AcOEt (**2e**, **2m**), pure hexane to 99 : 1 hexane-AcOEt (**2h**–**l**, **2n**–**r**) (isolated yields are shown in Table 2).

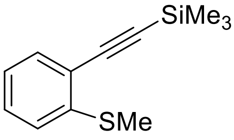
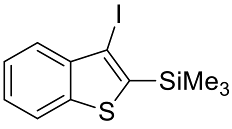
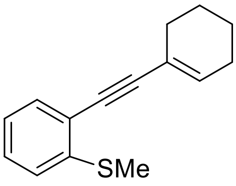
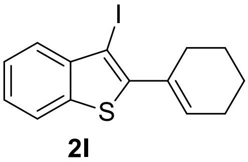
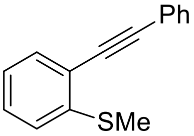
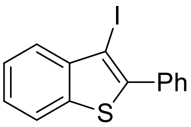
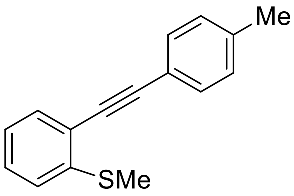
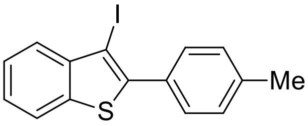
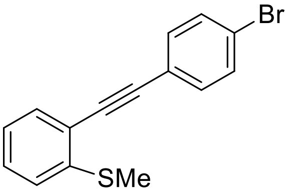
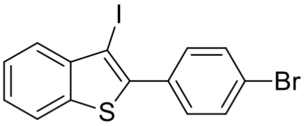
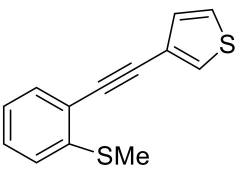
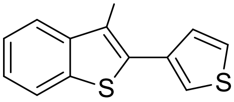
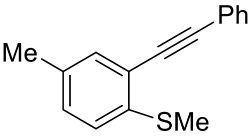
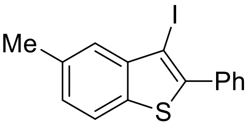
Recycling procedure: To the DES residue obtained as described above was added a solution of **1** (0.30 mmol), I_2 (0.60 mmol) and KI (0.60 mmol) in Et_2O (1.5 mL). The Et_2O was removed under vacuum and then the same procedure described above was followed.

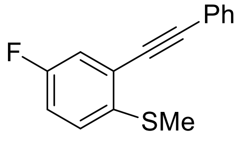
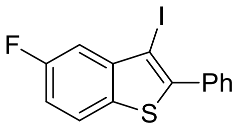
Table 2. Synthesis of 3-iodobenzothiophenes **2** by iodocyclization of 2-methylthiophenylacetylenes **1** in DES.[a]



Entry	**1**	**2**	Yield of **2**[%][b]
1	 **1a**	 **2a**	81
2	 **1b**	 **2b**	81 (80, 78, 78, 77, 75)
3	 **1c**	 **2c**	86

4	 **1d**	 **2d**	80
5	 **1e**	 **2e**	75
6	 **1f**	 **2f**	80
7	 **1g**	 **2g**	78
8	 **1h**	 **2h**	83
9	 **1i**	 **2i**	89 (86, 86, 85, 83, 82)
10	 **1j**	 **2j**	79

11	 **1k**	 **2k**	80
12	 **1l**	 **2l**	79
13	 **1m**	 **2m**	84 (83, 82, 82, 80, 80)
14	 **1n**	 **2n**	88
15	 **1o**	 **2o**	88 (87, 85, 83, 80, 80)
16	 **1p**	 **2p**	71
17	 **1q**	 **2q**	68

18	 **1r**	 **2r**	77

[a] All reactions were carried out in ChCl/urea (1/2, mol/mol) (0.15 mmol of **1** per mL of solvent) at 60 °C for 18 h in the presence of 2 equiv of I_2 and 2 equiv of KI. [b] Isolated yield based on starting **1**. Data in parentheses refer to the yields obtained after solvent recycles.

Representative iodocyclization procedure in larger scale: To a solution of **1a** (250 mg, 1.21 mmol) in ChCl/Urea (1 : 2, mol/mol; 8.0 mL) was added I_2 (610 mg, 2.4 mmol) and KI (400 mg, 2.4 mmol). The mixture was allowed to stir at 60 °C for 18 h and then extracted with Et_2O (6 × 3 mL) at 60 °C. After cooling, saturated aqueous $Na_2S_2O_3$ (25 mL) was added to the collected ethereal phases, and the mixture was allowed to stir for 10 min. Phases were separated, and the aqueous phase was extracted with Et_2O (3 × 20 mL). The collected organic layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, product **2a** was purified by column chromatography on silica gel using as eluent pure hexane to 9:1 hexane-AcOEt (yield: 309 mg, 80%).

2-(3-Iodobenzo[b]thiophen-2-yl)propan-2-ol (**2a**). Yield: 78.2 mg, starting from 62.5 mg of **1a** (81%) (Table 2, entry 1). Yellow solid, mp = 24–25 °C. IR (KBr): ν = 3410 (s, br), 1450 (m), 1435 (w), 1366 (m), 1242 (w), 1219 (m), 1165 (s), 1134 (s), 1018 (w), 949 (m), 895 (m), 756 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.77–7.71 (m, 2 H), 7.44–7.39 (m, 1 H), 7.36–7.30 (m, 1 H), 2.72 (s, 1 H), 1.83 (s, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 151.9, 142.7, 136.8, 125.3, 125.13, 125.08, 122.0, 73.8, 73.1, 29.9; GC-MS (EI, 70 eV): *m/z* = 318 (M^+, 55), 303 (100), 300 (42), 171 (11), 147 (18), 129 (23), 115 (14), 89 (31); HRMS- ESI (*m/z*): [$(M-H_2O+H)^+$] cald for $(C_{11}H_{10}IS)^+$: 300.9542; found: 300.9556.

2-(3-Iodobenzo[b]thiophen-2-yl)butan-2-ol (**2b**). Yield: 82.0 mg, starting from 67.0 mg of **1b** (81%) (Table 2, entry 2). Yellow oil. IR (film): ν = 3449 (s, br), 1451 (m), 1373 8 m), 1288 (w), 1242 (w), 1157 (s), 1034 (w), 995 (w), 918 (m), 756 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.77–7.72 (m, 2 H), 7.44–7.38 (m, 1 H), 7.36–7.31 (m, 1 H), 2.53 (s, 1 H), 2.45 (dq, *J* = 14.9, 7.4, 1 H), ;1.99 (dq, *J* = 14.6, 7.4, 1 H), 1.79 (s, 3 H), 0.90 (t, *J* = 7.4, 3 H) ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 151.5, 142.7, 137.1, 125.4, 125.0, 124.9, 122.0, 75.8, 73.4, 34.2, 28.3, 8.0; GC-MS (EI, 70 eV): *m/z* = 332 (M^+, 26), 303 (100), 161 (12), 147 (12), 89 (14); HRMS-ESI (*m/z*): [($M-H_2O+H)^+$] cald for $(C_{11}H_{12}IS)^+$: 314.9699; found: 314.9709.

1-(3-Iodobenzo[b]thiophen-2-yl)cyclohexan-1-ol (**2c**). Yield: 92.1 mg, starting from 73.5 mg of **1c** (86%) (Table 2, entry 3). Yellow oil. IR (film): ν= 3395 (m, br), 1443 (m), 1381 (w), 1319 (w), 1265 (w), 1242 (w), 1142 (m), 1065 (w), 1026 (m), 957 (s), 903 (m), 810 (m), 748 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.77 (d, *J* = 8.1, 1 H), 7.72 (d, *J* = 7.9, 1 H), 7.43–7.37 (m, 1 H), 7.31–7.29 (m, 1 H), 2.43 (s, 1 H), 2.40 (td, *J* = 13.4, 4.1, 2 H), 2.00–1.90 (m, 2 H), 1.84–1.63 (m, 5 H), 1.43– 1.30 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 151.9, 142.6, 136.8, 125.3, 125.1, 125.0, 122.0, 74.1, 73.4, 36.3, 25.0, 21.7; GC-MS (EI, 70 eV): *m/z* = 358 (M^+, 36), 240 (67), 315 (21), 302 (15), 287 (17), 231 (39), 213 (44), 188 (75), 185 (73), 171 (26), 147 (100), 115 (34). The spectroscopic data agreed with those reported.[8e]

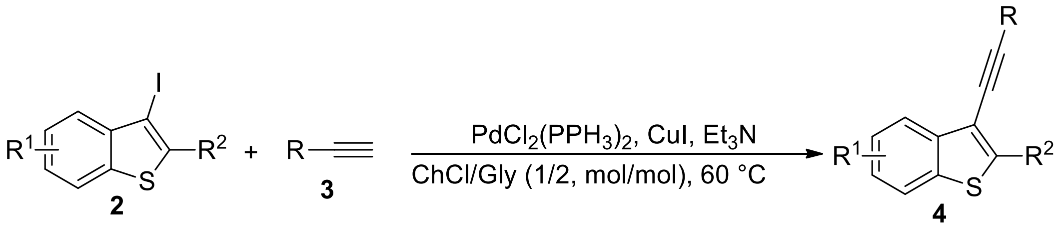
1-(3-Iodobenzo[b]thiophen-2-yl)cyclopentan-1-ol (**2d**). Yield: 83.6 mg, starting from 70.2 mg of **1d** (80%) (Table 2, entry 4). Brown oil. IR (film): ν= 3418 (s, br), 1435 (m), 1288 (w), 1242 (m), 1196 (m), 1003 (m), 756 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ=7.75 (d, *J*=8.3, 1 H), 7.73 (d, *J*=8.1, 1 H), 7.43‑7.38 (m, 1 H), 7.36 7.30 (m, 1 H), 2.53‑2.41 (m, 3 H), 2.22‑2.13 (m, 2 H), 2.06‑1.88 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ=149.3, 142.6, 136.8, 125.14, 125.11, 125.08, 122.0, 82.6, 74.9, 41.2, 24.0; GC-MS (EI, 70 eV): *m/z*=344 (M+, 70), 326 (100), 302 (29), 287 (38), 217 (66), 199 (34), 189 (42), 188 (48), 183 (45), 173 (28), 166 (26), 147 (72); HRMS-ESI (*m/z*): [(M-H2O+H)+] cald for $(C_{13}H_{12}IS)^+$: 326.9699; found: 326.9708.

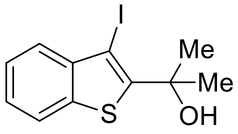
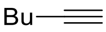
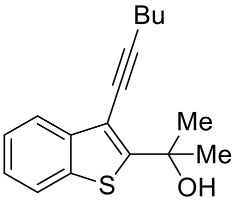
1-(3-Iodobenzo[b]thiophen-2-yl)ethan-1-ol (**2e**). Yield: 69.3 mg, starting from 58.2 mg of **1e**(75%) (Table 2, entry 5). Yellow oil. IR (film): ν = 3348 (s, br), 1435 (m), 1373 (w), 1319 (W), 1288 (w)m, 1250 (m), 1165 (m), 1096 (m), 1065 (s), 988 (w), 910 (w), 748 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.74 (d, *J* = 7.9, 1 H), 7.70 (d, *J* = 7.9, 1 H), 7.40 (t, *J* = 7.5, 1 H), 7.33 (t, *J* = 7.5, 1 H), 5.37–5.30 (m, 1 H), 2.69 (s, br, 1 H), 1.60 (d, *J* = 6.4, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 148.8, 140.8, 137.4, 125.3, 125.2, 125.0, 122.6, 76.8, 69.2, 24.0; GC-MS (EI, 70 eV): *m/z* = 304 (M^+, 30), 289 (29), 286 (32), 159

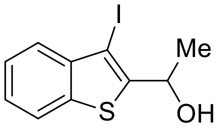
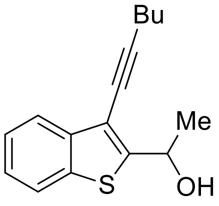
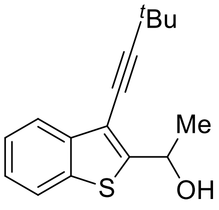
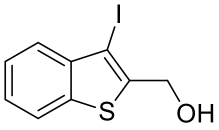
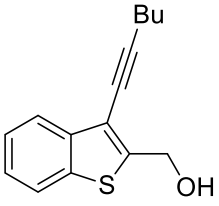
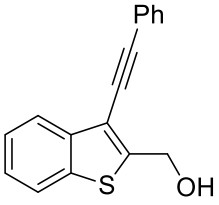
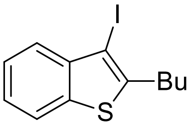
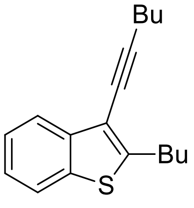
(20), 134 (98), 115 (100), 89 (21); HRMS ESI (*m/z*): [(M-H_2O+H)$^+$] cald for ($C_{10}H_{18}IS$)$^+$: 286.9386; found: 286.9398.

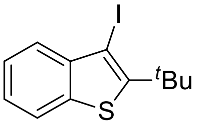
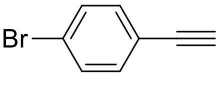
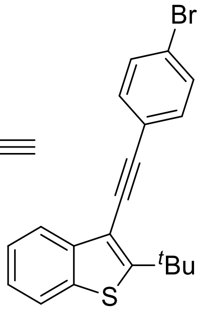
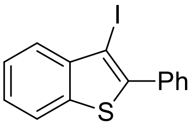
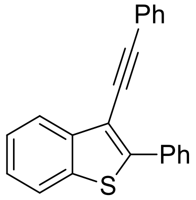
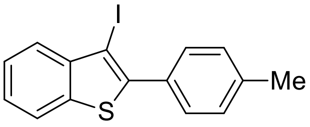
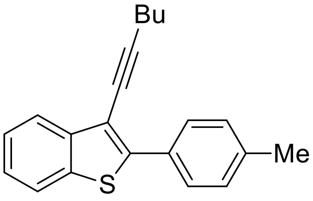
1-(3-Iodobenzo[b]thiophen-2-yl)(phenyl)methanol (**2f**). Yield: 87.8 mg, starting from 76.0 mg of **1f** (80%) (Table 2, entry 6). Yellow oil. IR (film): ν = 1643 (w), 1597 (w), 1489 (w), 1450 (m), 1273 (m), 1250 (m), 1188 (w), 1088 (s), 1026 (w), 972 (w), 910 (w), 756 (s), 702 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.74–7.70 (m, 2 H), 7.53–7.51 (m, 2 H), 7.42–7.37 (m, 1 H), 7.37–7.26 (m, 4 H), 6.30 (s, 1 H), 2.63 (s, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 146.9, 141.4, 140.8, 138.0, 128.6, 128.3, 126.6, 125.5, 125.3, 125.2, 122.6, 79.3, 74.6; GC-MS (EI, 70 eV): *m/z* = 366 (M$^+$, 27), 237 (9), 221 (8), 161 (8), 134 (16), 105 (100). The spectroscopic data agreed with those reported.[8f]

Table 3. Synthesis of 3-alkynylbenzothiophenes **4** by Sonogashira coupling of 3-iodobenzothiophenes **2** with 1-alkynes **3** in DES.[a]



Entry	**2**	**3**	**4**	Yield of **4**[%][b]
1[c]	 **2a**	 **3a**	 **4aa**	48
2	2a	3a	4aa	77

3	 **2e**	3a	 **4ea**	75
4	2e	^{t}Bu—≡ **3b**	 **4eb**	81
5	 **2g**	3a	 **4ga**	76
6	2g	Ph—≡ **3c**	 **4gc**	84 (83, 84, 83, 83, 83)
7	 **2i**	3a	 **4ia**	80

8	 **2j**	 **3d**	 **4jd**	59
9	 **2m**	3c	 **4mc**	87 (86, 87, 85, 85, 86)
10	 **2n**	3a	 4na	74

[a] Unless otherwise noted, all reactions were carried out in ChCl/Gly (1/2, mol/mol) (0.17 mmol of **2** per mL of solvent) at 60 °C for 15 h, in the presence of 5 mol% of $PdCl_2(PPh_3)_2$, 20 mol% of CuI, 3 equiv. of Et_3N and 2.4 equiv. of 1-alkyne **3**. [b] Isolated yield based on starting **2**. Data in parentheses refer to the yields obtained after the recycling of the solvent-catalyst system [DES containing $PdCl_2(PPh_3)_2$ and CuI]. [c] The reaction was carried out using ChCl/urea (1/2, mol/mol) as the solvent.

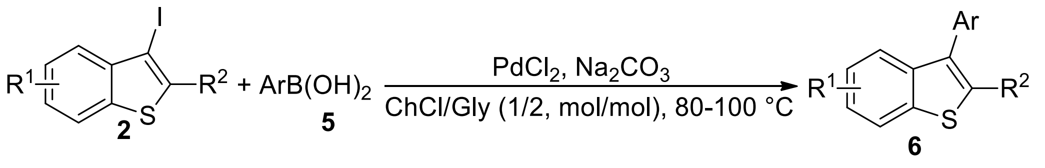
(3-Iodobenzo[b]thiophen-2-yl)methanol (**2g**). Yield: 68.4 mg, starting from 53.7 mg of **1g** (78%) (Table 2, entry 7). Yellow oil. IR (film): ν= 3264 (m, br), 1481 (w), 1450 (w), 1427 (m), 1342 (m), 1242 (w), 1150 (m), 1034 (s), 903 (w), 748 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ= 7.75 (d, J = 7.9, 1 H), 7.70 (d, J = 8.2, 1 H), 7.44–7.39 (m, 1 H), 7.37–7.32 (m, 1 H), 4.93 (d, J = 5.2, 2 H), 2.46–2.41 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 143.0, 140.9, 138.3, 125.5, 125.3, 125.2, 122.6, 78.9, 62.9; GC-MS (EI, 70 eV): m/z = 290 (M^+, 72), 273 (7), 163 (28), 135 (100), 102 (12), 91 (66). The spectroscopic data agreed with those reported.[8n]

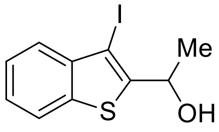
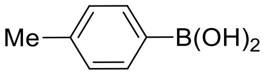
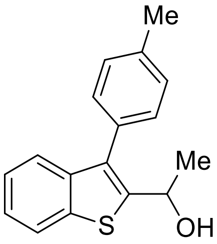
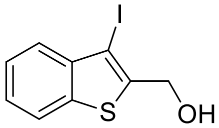
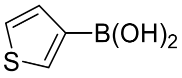
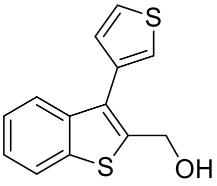
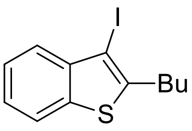
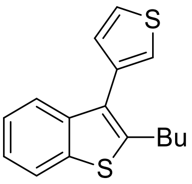
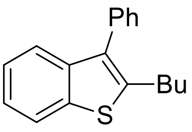
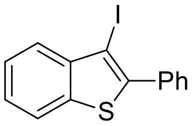
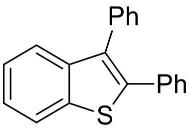
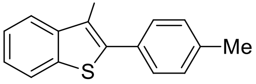
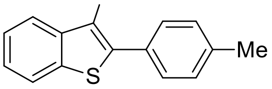
3-Iodo-2-phenethylbenzo[b]thiophene (**2h**). Yield: 91.2 mg, starting from 76.0 mg of **1h** (83%) (Table 2, entry 8). Colorless oil. IR (film): ν = 1605 (m), 1497 (m), 1450 (m), 1435 (s), 1250 (m), 1157 (w), 1111 (w), 1072 (w), 1018 (m), 903 (m), 748 (s), 703 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.71–7.66 (m, 2 H), 7.41 7.36 (m, 1 H), 7.32–7.26 (m, 3 H), 7.26–7,18 (m, 3 H), 3.27–3.21 (m, 2 H), 3.04 2.98 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 143.2, 141.1, 140.4, 138.1, 128.51, 128.47, 126.3, 125.1, 124.9, 122.2, 80.6, 36.6, 34.9; GC-MS (EI, 70 eV): *m/z* = 364 (M$^+$, 46), 273 (100), 237 (41), 146 (22); HRMS-ESI (*m/z*): [(M+ H)$^+$] cald for $(C_{16}H_{14}IS)^+$: 364.9855; found: 364.9870.

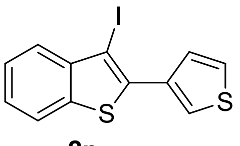
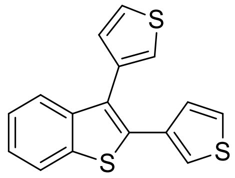
2-Butyl-3-iodobenzo[b]thiophene (**2i**). Yield: 85.0 mg, starting from 61.5 mg of **1i** (89%) (Table 2, entry 9). Colorless oil. IR (film): ν= 1458 (m), 1435 (m), 1381 (w), 1296 (w), 1250 (m), 1072 (w), 1018 (w), 903 (m), 748 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.68 (t, *J* = 8.6, 2 H), 7.37 (t, *J* = 7.3, 1 H), 7.28 (t, *J* = 7.3, 1 H), 2.94 (t, *J* = 7.5, 2 H), 1.71 (quintuplet, *J* = 7.5, 2 H), 1.43 (hexuplet, *J* = 7.5, 2 H), 0.96 (t, *J* = 7.5, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 144.7, 141.1, 138.0, 125.0, 124.7, 122.1, 80.1, 32.6, 29.7, 22.2, 13.9; GC-MS (EI, 70 eV): *m/ z* = 316 (M$^+$, 55), 273 (93), 147 (100), 115 (17), 102 (21). The spectroscopic data agreed with those reported.[8f]

2-(tert-Butyl)-3-iodobenzo[b]thiophene (**2j**). Yield: 75.7 mg, starting from 61.7 mg of **1j** (79%) (Table 2, entry 10). Colorless oil. IR (film): ν = 1458 (m), 1427 (m), 1366 (w), 1234 (m), 1026 (w), 895 (w), 849 (w), 748 (s), 725 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.78 (d, *J* = 8.3, 1 H), 7.69 (d, *J* = 7.9, 1 H), 7.40–7.35 (m, 1 H), 7.31–7.26 (m, 1 H), 1.61 (s, 9 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 151.7, 142.8, 136.1, 125.2, 124.9, 124.7, 121.6, 74.7, 35.7, 30.2; GC-MS (EI, 70 eV): *m/z* = 316 (M$^+$, 82), 301 (100), 273 (30), 174 (85), 147 (55), 134 (40), 115 (91). The spectroscopic data agreed with those reported.[8f]

Table 4. Synthesis of arylbenzothiophenes **6** by Suzuki coupling of 3-iodobenzothiophenes **2** with arylboronic acids **5** in DES.[a]



Entry	**2**	**5**	T [°C]	*t* [h]	**6**	Yield of **6** [%][b]
1	 **2e**	 **5a**	80	15	 **6ea**	95 (94, 95, 94, 94, 93)
2	 **2g**	 **5b**	80	15	 **6gb**	92
3	 **2i**	5b	100	24	 **6ib**	70
4	2i	PhB(OH)$_2$ **5c**	100	24	 **6ic**	68
5	 **2m**	5c	80	15	 **6mc**	73 (72, 73, 71, 71, 71)
6	 **2n**	5c	80	15	 **6nc**	69

7	 **2p**	5b	80	15	 **6pb**	80 (80, 79, 80, 80, 79)

[a] All reactions were carried out in ChCl/Gly (1/2, mol/mol) (0.17 mmol of **2** per mL of solvent), in the presence of 10 mol% of $PdCl_2$, 2 equiv. of Na_2CO_3 and 1.5 equiv. of arylboronic acid **5**. [b] Isolated yield based on starting **2**. Data in parentheses refer to the yields obtained after the recycling of the solvent-catalyst system (DES containing $PdCl_2$).

(3-Iodobenzo[b]thiophene-2-yl)trimethylsilane (**2k**). Yield: 80.1 mg, starting from 66.2 mg of **1k** (80%) (Table 2, entry 11). Colorless oil. IR (film): ν= 1466 (m), 1412 (w), 1250 (s), 1157 (w), 1072 (w), 1018 (w), 972 (s), 841 (s), 756 (s), 725 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ= 7.85–7.73 (m, 2 H), 7.45–7.37 (m, 1 H), 7.37–7.28 (m, 1 H), 0.50 (s, 9 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 143.1, 141.4, 140.8, 125.4, 125.2, 125.0, 121.9, 87.1, 0.61; GC-MS (EI, 70 eV): *m/z* = 332 (M^+, 95), 317 (100), 189 (43), 175 (26), 158 (9), 147 (18), 115 (50). The spectroscopic data agreed with those reported.[8f]

2-(Cyclohex-1-en-1-yl)-3-iodobenzo[b]thiophene (**2l**). Yield: 80.3 mg, starting from 68.2 mg of **1l** (79%) (Table 2, entry 12). Colorless oil. IR (film): ν= 1435 (s), 1342 (w), 1242 (m), 1165 (w), 1134 (w), 1072 (w), 980 (w), 748 (s), 725 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ= 7.76–7.72 (m, 1 H), 7.72–7.68 (m, 1 H), 7.42–7.37 (m, 1 H), 7.33–7.28 (m, 1 H), 6.20–6.16 (m, 1 H), 2.48–2.42 (m, 2 H), 2.27–2.21 (m, 2 H), 1.83–1.76 (m, 2 H), 1.73–1.67 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 145.1, 141.5, 137.9, 132.5, 132.2, 125.7, 125.1, 125.0, 122.0, 77.4, 29.9, 25.6, 22.8, 21.7; GC-MS (EI, 70 eV): *m/z* = 340 (M^+, 100), 312 (5), 213 (22), 185 (49), 171 (14), 147 (31), 79 (26). The spectroscopic data agreed with those reported.[8f]

3-Iodo-2-phenylbenzo[b]thiophene (**2m**). Yield: 85.3 mg, starting from 67.5 mg of **1m** (84%) (Table 2, entry 13). Yellow solid, mp = 55–56 °C. IR (KBr): ν= 1481 (m), 1435 (s), 1242 (s), 1157 (w), 1072 (w), 1026 (w), 871 (w), 748 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ= 7.83 (d, *J* = 7.9, 1 H), 7.77 (d, *J* = 7.9, 1 H), 7.68 (d, *J* = 7.5, 2 H), 7.51–7.41 (m, 4 H), 7.38 (dist t, *J* = 7.5, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 142.2, 141.9, 138.9, 134.6, 130.0, 128.9, 128.5, 126.3, 125.48, 125.45, 122.1, 79.4; GC-MS (EI, 70 eV): *m/z*

= 336 (M^+, 100), 208 (39), 165 (41), 104 (27). The spectroscopic data agreed with those reported.[14]

3-Iodo-2-(p-tolyl)benzo[b]thiophene (**2n**). Yield: 92.5 mg, starting from 71.6 mg of **1n** (88%) (Table 2, entry 14). Yellow oil. IR (film): ν = 1489 (m), 1435 (s), 1246 (m), 1111 (w), 1018 (w), 964 (w), 879 (w), 818 (m), 795 (m), 748 (s), 725 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ= 7.83–7.79 (m, 1 H), 7.75 (d, *J* = 8.0, 1 H), 7.59–7.55 (m, 2 H), 7.46–7.42 (m, 1 H), 7.38–7.33 (m, 1 H), 7.24 (d, *J* = 8.2, 2 H), 2.41 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 142.3, 141.9, 139.0, 138.9, 131.7, 129.9, 129.2, 126.2, 125.4, 125.38, 125.36, 122.1, 79.1, 21.4; GC-MS (EI, 70 eV): *m/z* = 350 (M^+, 100), 221 (21), 208 (34), 179 (21), 111 816).[8f]

2-(4-Bromophenyl)3-iodobenzo[b]thiophene (**2o**). Yield: 109.0 mg, starting from 90.4 mg of **1o** (88%) (Table 2, entry 15). Yellow oil. IR (film): ν = 1474 (m), 1427 (w), 1389 (w), 1242 (w), 1065 (m), 1011 (m), 964 (w), 826 (s), 772 (m), 748 (s), 718 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ= 7.81 (d, *J* = 8.0, 1 H), 7.77 (d, *J* = 7.9, 1 H), 7.64–7.57 (m, 2 H), 7.57–7.51 (m, 2 H), 7.46 (t, *J* = 7.5, 1 H), 7.39 (t, *J* = 7.5, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ= 141.8, 140.8, 138.9, 133.6, 131.8, 131.5, 126.4, 125.7, 125.6, 123.3, 122.1, 79.9; GC-MS (EI, 70 eV): *m/z* = 416 [$(M+2)^+$, 46], 414 (M^+, 53), 208 (100), 176 (3), 163 (37), 104 (45); HRMS-ESI (*m/z*): [$(M+H)^+$] cald for $(C_{14}H_9BrIS)^+$: 414.8648; found: 414.8675. The spectroscopic data agreed with those reported.[8a]

3-Iodo-2-(thiophen-3-yl)benzo[b]thiophene (**2p**). Yield: 73.2 mg, starting from 69.2 mg of **1p** (71%) (Table 2, entry 16). Yellow solid, mp= 40–41 °C. IR (KBr): ν = 1435 (m), 1358 (w), 1242 (m), 1165 (w), 1080 (w), 1018 (w), 910 (w), 849 (m), 779 (s), 748 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ= 7.90–7.67 (m, 3 H), 7.57–7.48 (m, 1 H), 7.48–7.27 (m, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 142.1, 138.2, 137.3, 134.7, 128.4, 126.2, 125.8, 125.5, 125.4, 122.0, 78.6; GC-MS (EI, 70 eV): *m/z* = 342 (M^+, 100), 215 (20), 214 (19), 171 (78), 127 (12), 107 (25); HRMS-ESI (*m/z*): [$(M+H)^+$] cald for $(C_{12}H_8IS_2)^+$: 342.9107; found: 342.9144.

3-Iodo-5-methyl-2-phenylbenzo[b]thiophene (**2q**). Yield: 71.5 mg, starting from 71.6 mg of **1q** (68%) (Table 2, entry 17). Yellow solid, mp = 55–56 °C. IR (KBr): ν = 1605 (w), 1443 (s), 1381 (w), 1250 (w), 1157 (w), 1072 (w), 802 (m), 764 (m), 741 (m) cm^{-1};

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.69–7.64 (m, 4 H), 7.48–7.39 (m, 3 H), 7.22–7.18 (m, 1 H), 2.52 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 142.3, 142.1, 136.1, 135.4, 134.8, 130.0, 128.8, 138.5, 127.2, 126.2, 121.8, 79.1, 21.5; GC-MS (EI, 70 eV): *m/z* = 350 (M^+, 100), 221 (20), 208 (34), 179 (18), 11); HRMS-ESI (*m/z*): [$(M+H)^+$] cald for $(C_{15}H_{12}IS)^+$: 350.9699; found: 350.9670.

5-Fluoro-3-Iodo-2-phenylbenzo[b]thiophene (**2r**). Yield: 81.8 mg, starting from 72.8 mg of **1r** (77%) (Table 2, entry 18). Yellow solid, mp= 55–56 °C. IR (KBr): ν= 1597 (m), 1566 (w), 1443 (s), 1273 (m), 1234 (w), 1165 (m), 1126 (w), 988 (w), 856 (m), 802 (m), 741 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.71 (dd, *J* = 8.7, 4.8, 1 H), 7.68–7.63 (m, 2 H), 7.52 (dd, *J* = 9.6, 2.3, 1 H), 7.50–7.42 (m, 3 H), 7.13 (td, *J* = 8.7, 2.3, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 161.7 (d, *J* = 242.5), 144.8, 143.5 (d, *J* = 8.9), 134.4 (d, *J* = 8.9), 129.9, 129.1, 128.6, 123.4 (d, *J* = 8.8), 114.3 (d, *J* = 25.1), 112.1 (d, *J* = 24.6), 74.5; GC-MS (EI, 70 eV): *m/z* = 354 (100), 226 (45), 183 (39), 113 (31); HRMS-ESI (*m/z*): [$(M +H)^+$] cald for $(C_{14}H_9FIS)^+$: 354.9448; found: 354.9429. The spectroscopic data agreed with those reported.[8a]

Sonogashira coupling leading to 3-alkynylbenzothiophenes 4 in DES (Table 3): To a solution of 3-iodobenzothiophenes **2** (0.25 mmol; **2a**, 80.0 mg; **2e**, 76.3 mg; **2g**, 72.8 mg; **2i**, 79.2 mg; **2j**, 80.0 mg; **2m**, 84.2 mg; **2n**, 87.8 mg) in ChCl/Gly (1 : 2, mol/mol;

1.5 mL) were added $PdCl_2(PPh_3)_2$ (8.8 mg, 1.25×10^{-2} mmol), CuI (9.5 mg, 0.05 mmol), the terminal alkyne (0.6 mmol; 1-hexyne **3a**, 50.0 mg; 3,3-dimethyl-1-butyne **3b**, 49.8 mg; phenylacetylene **3c**, 61.5 mg; 1-bromo-4-ethynylbenzene **3d**, 108.2 mg), and anhydrous Et_3N (105 μL, 76.2 mg, 0.75 mmol). The mixture was allowed to stir at 60 °C (oil bath) for 15 h. After cooling, the mixture was extracted with Et_2O (6 × 5 mL) at 60 °C, and the DES phase used again for the recycling experiments (see below). After evaporation of the solvent from the collected ethereal phases, products **4** were purified by column chromatography on silica gel using as eluent pure hexane to 95 : 5 hexane-AcOEt (**4aa**, **4ga**, **4gc**), pure hexane to 99 : 1 hexane-AcOEt (**4ea**, **4ia**, **4jd**, **4mc**, **4na**), hexane to 95 : 5 hexane- Et_2O (**4eb**) (isolated yields are shown in Table 3).

Recycling procedure: To the DES residue obtained as described above, still containing the catalysts $PdCl_2(PPh_3)_2$ and CuI, was added a solution of **2** (0.25 mmol) in Et_2O (1.5

mL). The Et_2O was removed under vacuum and the terminal alkyne **3** (0.6 mmol) followed by anhydrous Et_3N (105 µL, 0.75 mmol) were added. Then the same procedure described above was followed.

Representative Sonogashira coupling procedure in larger scale: To a solution of 2-(3-iodobenzo[*b*]thiophen-2-yl)propan-2-ol **2a** (1.0 mmol; 320.0 mg) in ChCl/Gly (1 : 2, mol/mol; 6 mL) were added $PdCl_2(PPh_3)_2$ (35.3 mg, 0.05 mmol), CuI (38.2 mg, 0.2 mmol), 1-hexyne (200.2 mg, 2.44 mmol), and anhydrous Et_3N (420 µL, 304.9 mg, 3.0 mmol). The mixture was allowed to stir at 60 °C (oil bath) for 15 h. After cooling, the mixture was extracted with Et_2O (6 × 8 mL) at 60 °C. After evaporation of the solvent from the collected ethereal phases, product **4aa** was purified by column chromatography on silica gel using as eluent pure hexane to 95 : 5 hexane- AcOEt (yield: 220 mg, 81%).

2-(3-(Hex-1-yn-1-yl)benzo[b]thiophen-2-yl)propan-2-ol (**4aa**). Yield: 53.0 mg, starting from 80.0 mg of **2a** (77%) (Table 3, entry 2). Yellow oil. IR (film): ν = 2214 (vw), 1597 (w), 1489 (m), 1462 (m), 1435 (m), 1254 (w), 1069 (m), 752 (s), 667 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ = 7.84–7.78 (m, 1 H), 7.76–7.71 (m, 1 H), 7.41–7.35 (m, 1 H), 7.34–7.27 (m, 1 H), 3.04 (s, br, 1 H), 2.55 (t, *J* = 7.0, 2 H), 1.71–1.62 (m, 2 H), 1.57–1.48 (m, 1 H), 0.98 (t, *J* = 7.3, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 157.0, 141.4, 136.4, 131.6, 130.4, 124.5, 122.5, 122.1, 112.3, 98.1, 73.9, 72.9, 30.9, 30.4, 22.1, 19.4, 13.6; GC-MS (EI, 70 eV): *m/z* = 272 (M^+, 74), 257 (100), 254 (16), 238 (31), 225 (16), 211 (58), 197 (39), 185 (36), 184 (36), 171 (31), 165 (16), 152 (20), 147 (14), 139 (14), 147 (14), 139 (14), 127 (13), 115 (13); HRMS-ESI (*m/z*): [$(M\text{-}H_2O+H)^+$] cald for $(C_{17}H_{19}S)^+$: 255.1202; found: 255.1213.

1-(3-(Hex-1-yn-1-yl)benzo[b]thiophen-2-yl)ethan-1-ol (**4ea**). Yield: 48.7 mg, starting from 76.3 mg of **2e** (75%) (Table 3, entry 3). Yellow oil. IR (film): ν = 3356 (s, br), 1458 (m), 1435 (w), 1366 (w), 1319 (w), 1188 (m), 1096 (m), 1065 (s), 756 (s), 733 (m) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ= 7.82 (d, *J* = 7.6, 1 H), 7.75 (d, *J* = 7.6 1 H), 7.37 (t, *J* = 7.6, 1 H), 7.31 (t, *J* = 7.6, 1 H), 5.74 (q, *J* = 6.3, 1 H), 2.52 (t, J= 7.0, 2 H), 2.41 (s, br, 1H), 1.72–1.45 (m, 8 H), 0.98 (t, *J* = 7.3, 1 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 153.0, 140.3, 137.4, 124.8, 124.6, 122.8, 122.5, 114.7, 97.1, 73.1, 66.3, 31.1, 24.2, 22.1, 19.4, 13.6; GC-MS (EI, 70 eV): *m/z* = 258 (M^+, 31), 240 (53), 225 (16), 211 (34), 198 (48), 197

(100), 184 (31), 178 (22), 171 (27), 165 (34); HRMS-ESI (*m/z*): $[(M\text{-}H_2O+H)^+]$ cald for $(C_{16}H_{17}S)^+$: 241.1045; found: 241.1056.

1-(3-(3,3-Dimethylbut-1-yn-1-yl)benzo[b]thiophen-2-yl)ethan-1-ol (**4eb**). Yield: 52.5 mg, starting from 76.0 mg of **2e** (81%) (Table 3, entry 4). Yellow oil. IR (film): ν = 3356 (s, br), 1450 (m), 1366 (m), 1319 (w), 1250 (m), 1188 (m), 1096 (s), 756 (m), 733 (m); ^{1}H NMR (CDCl$_3$, 500 MHz): δ= 7.82–7.77 (m, 1H), 7.78–7.71 (m, 1 H), 7.41–7.35 (m, 1H), 7.34 (m, 1H), 5.46 (q, *J* = 6.4, 1 H), 2.67 (s, br, 1H), 1.63 (d, *J* = 6.5, 3H), 1.38 (s, 9 H); ^{13}C NMR (CDCl$_3$, 125 MHz): δ= 152.8, 140.0, 137.3, 124.8, 124.5, 122.7, 122.5, 114.3, 105.5, 71.5, 66.2, 34.0, 28.4, 24.1; GC-MS (EI, 70 eV): *m/z* = 258 (M$^+$, 100), 243 (43), 240 (34), 227 (32), 215 (46), 227 (28), 201 (98), 184 (48), 161 (28); HRMS-ESI (*m/z*): $[(M\text{-}H2O+H)^+]$ cald for $(C^{16}H^{17}S)^+$: 241.1045; found: 241.1055.

(3-(Hex-1-yn-1-yl)benzo[b]thiophen-2-yl)methanol (**4ga**). Yield: 46.8 mg, starting from 72.8 mg of **2g** (76%) (Table 3, entry 5). Yellow oil. IR (film): ν = 2222 (vw), 1458 (m), 1435 (m), 1188 (m), 1126 (w), 1026 (s), 756 (m), 733 (m) cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz): δ= 7.88–7.81 (m, 1H), 7.80–7.72 (m, 1H), 7.43–7.36 (m, 1H), 7.36– 7.30 (m, 1H), 2.51 (t, *J* = 7.0, 2 H), 2.39 (s, br, 1H), 1.70–1.61 (m, 2H), 1.59–1.48 (m, 2H), 0.97 (t, *J* = 7.3, 3H); ^{13}C NMR (CDCl$_3$, 125 MHz): δ= 146.8, 139.9, 138.1, 125.0, 124.6, 123.0, 122.4, 116.2, 96.7, 72.8, 59.7, 30.9, 22.0, 19.3, 13.6; GC-MS (EI, 70 eV): *m/z* = 244 (M$^+$, 100), 225 (18), 211 (21), 201 (27), 187 (32), 171 (29), 147 (30), 129 (23), 115 (14); HRMS-ESI (*m/z*): $[(M\ H_2O+\ H)^+]$ cald for $(C_{15}H_{15}S)^+$: 227.0889; found: 227.0897.

(3-(Phenylethynyl)benzo[b]thiophen-2-yl)methanol (**4gc**). Yield: 56.0 mg, starting from 73.0 mg of **2g** (84%) (Table 3, entry 6). Yellow solid, mp = 138–140 °C. IR (KBr): ν = 2206 (vw), 1597 (w), 1435 (m), 1381 (m), 1219 (w), 1157 (w), 1026 (m), 772 (s) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ= 8.01 (d, *J* = 7.9, 1H), 7.93 (d, *J* = 7.9, 1H), 7.67–7.63 (m, 2H), 7.53–7.38 (m, 5H), 5.94 (t, *J* = 5.6, 1H), 5.00 (d, *J* = 5.6, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 152.6, 139.0, 137.2, 131.4, 128.80, 128.75, 125.03, 124.97, 122.9, 122.3, 122.1, 112.2, 95.0, 81.7, 58.2; GC-MS (EI, 70 eV): *m/z* = 264 (M$^+$, 100), 235 (64), 234 (52), 202 (23), 186 (17); HRMS-ESI (*m/z*): $[(M\text{-}H_2O+H)^+]$ cald for $(C_{17}H_{11}S)^+$: 247.0576; found: 247.0587.

2-Butyl-3-(hex-1-yn-1-yl)benzo[b]thiophene (**4ia**). Yield: 54.5 mg, starting from 79.2 mg of **2i** (80%) (Table 3, entry 7). Yellow oil. IR (film): ν = 2183 (vw), 1458 (m), 1435 (m), 1373 (m), 1319 (w), 1180 (w), 1065 (w), 756 (m), 733 (m), cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz): δ= 7.82–7.77 (m, 1H), 7.73–7.68 (m, 1H), 7.39–7.33 (m, 1H), 7.30–7.24 (m, 1H), 3.02 (t, *J* = 7.6, 2H), 2.53 (m, 2H), 1.77–1.69 (m, 2H), 1.69–1.61 (m, 2H), 1.59–1.50 (m, 2H), 1.47–1.38 (m, 2H), 0.98 (t, *J* = 7.3, 3H), 0.96 (t, *J* = 7.4, 3H); ^{13}C NMR (CDCl$_3$, 125 MHz): δ= 149.3, 140.3, 137.4, 124.4, 124.1, 122.4, 122.0, 115.7, 95.3, 73.8, 33.1, 31.1, 29.5, 22.2, 22.0, 19.4, 13.8, 13.6; GC-MS (EI, 70 eV): *m/z* = 270 (M$^+$, 96), 227 (100), 199 (18), 185 (61), 171 (21), 147 (34); HRMS-ESI (*m/z*): [(M+H)$^+$] cald for (C$_{18}$H$_{23}$S)$^+$: 271.1515; found: 271.1512.

3-((4-Bromophenyl)ethynyl)-2(tert-butyl)benzo[b]thiophene (**4jd**). Yield: 55.2 mg, starting from 80.0 mg of **2j** (59%) (Table 3, entry 8). Yellow solid, mp= 68–70 °C. IR (KBr): ν = 2207 (w), 1474 (m), 1358 (w), 1234 (w), 1204 (w), 1065 (m), 1011 (m), 818 (s), 756 (s), 725 (s) cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz): δ= 7.90 (d, *J* = 7.9, 1H), 7.75 (d, *J* = 7.9, 1H), 7.53–7.47 (m, 2H), 7.46–7.42 (m, 2H), 7.42–7.38 (m, 1H), 7.33 (t, *J* = 7.5, 1H), 1.63 (s, 9H, *t*-Bu); ^{13}C NMR (CDCl$_3$, 125 MHz): δ= 160.6, 141.3, 135.9, 133.8, 132.6, 131.7, 127.7, 124.6, 124.4, 122.2, 121.9, 112.3, 95.0, 85.3, 35.9, 30.7; GC-MS (EI, 70 eV): *m/z* = 370 [(M+ 2)$^+$, 39], 368 (36, M$^+$), 355 (28), 353 (27), 274 (100), 258 (30), 129 (22); HRMS-ESI (*m/z*): [(M +H)$^+$] cald for (C$_{20}$H$_{18}$BrS)$^+$: 369.0307; found: 369.0316.

2-Phenyl-3-(phenylethynyl)benzo[b]thiophene (**4mc**). Yield: 67.8 mg, starting from 84.2 mg of **2m** (87%) (Table 3, entry 9). Yellow solid, mp= 70–71 °C. IR (KBr):ν= 2199 (vw), 1597 (w), 1481 (m), 1435 (m), 1219 (m), 1072 (w), 1018 (w), 949 (w), 756 (s), 687 (m) cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz): δ= 8.07–8.03 (m, 2H), 8.02 (d, *J* = 8.0, 1H), 7.78 (d, *J* = 8.0, 1H), 7.58–7.54 (m, 2H), 7.47–7.40 (m, 3H), 7.40–7.29 (m, 5H); ^{13}C NMR (CDCl$_3$, 125 MHz): δ= 146.2, 141.0, 137.6, 133.9, 131.6, 128.8, 128.7, 128.43, 128.35, 125.25, 124.9, 123.4, 123.3, 122.1, 113.6, 94.7, 84.1; GC-MS (EI, 70 eV): *m/z* = 310 (M$^+$, 100), 309 (67), 308 (74), 276 (9), 154 (22); HRMS-ESI (*m/z*): [(M+ H)$^+$] cald for (C$_{22}$H$_{15}$S)$^+$: 311.0889; found: 311.0892.

3-(Hex-1-yn-1-yl)-2(p-tolyl)benzo[b]thiophene (**4na**). Yield: 56.6 mg, starting from 87.8 mg of **2n** (74%) (Table 3, entry 10). Yellow oil. IR (film): ν = 2214 (vw), 1597 (w), 1489 (m), 1462 (m), 1435 (m), 1254 (w), 1069 (m), 752 (s), 667 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.94–7.87 (m, 3H), 7.75 (d, *J* = 7.9), 7.40 (t, *J* = 7.5, 1 H), 7.32 (t, *J* = 7.4, 1H), 7.24 (d, *J* = 7.9, 2H), 2.54 (t, *J* = 7.0, 2H), 2.39 (s, 3H), 1.71–1.60 (m, 2H), 1.59-1.49 (m, 2H), 0.98 (t, *J* = 7.3, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 145.0, 141.6, 138.5, 137.4, 131.3, 129.3, 128.2, 124.9, 124.7, 123.2, 121.9, 113.9, 96.0, 75.1, 30.9, 22.1, 21.3, 19.5, 13.7; GC-MS (EI, 70 eV): *m/z* = 304 (M^+, 67), 289 (7), 261 (100), 247 (30), 245 (28), 202 (7); HRMS-ESI (*m/z*): [$(M+H)^+$] cald for $(C_{21}H_{21}S)^+$: 305.1358; found: 305.1364.

Suzuki coupling leading to 3-arylbenzothiophenes 6 in DES (Table 4): To a solution of 3-iodobenzothiophenes **2** (0.25 mmol; **2e**, 76.3 mg; **2g**, 72.8 mg; **2i**, 79.3 mg; **2m**, 84.2 mg; **2n**, 87.9 mg; **2p**, 85.8 mg) in ChCl/Gly (1 : 2, mol/mol; 1.5 mL) were added $PdCl_2$ (4.5 mg, 2.5 × 10^{-2} mmol), Na_2CO_3 (53.0 mg, 0.5 mmol), and the boronic acid (0.375 mmol; *p*-tolylboronic acid **5a**, 51.0 mg; thio- phen-3-ylboronic acid **5b**, 48.0 mg; phenylboronic acid **5c**, 45.8 mg). The reaction mixture was allowed to stir at 80 °C (oil bath) for 15 h (for **6ea**, **6gb**, **6mc**, **6nc**, and **6pb)**, or 100 °C (oil bath) for 24 h (for **6ib** and **6ic**). After cooling, the mixture was extracted with Et_2O (6 × 2 mL) at 60 °C, and the DES phase used again for the recycling experiments (see below). After evaporation of the solvent from the collected ethereal phases, products **6** were purified by column chromatography on silica gel using hexane to 99 : 1 hexane- AcOEt as the eluent (isolated yields are shown in Table 4).

Recycling procedure: To the DES residue obtained as described above, still containing $PdCl_2$, was added a solution of **2** (0.25 mmol) in Et_2O (1.5 mL). The Et_2O was removed under vacuum, then Na_2CO_3 (0.5 mmol) and the boronic acid **5** (0.375 mmol) were added. Then the same procedure described above was followed.

Representative Suzuki coupling procedure in larger scale: To a solution of (3-iodobenzo[*b*]thiophen-2-yl)methanol **2e** (305.0 mg, 1.00 mmol) in ChCl/Gly (1 : 2, mol/mol; 6 mL) were added $PdCl_2$ (18.0 mg, 0.1 mmol), Na_2CO_3 (212.0 mg, 2.0 mmol), and *p*-tolylboronic acid **5a** (204.0 mg, 1.5 mmol). The reaction mixture was allowed to stir at 80 °C (oil bath) for 15 h. After cooling, the mixture was extracted with Et_2O (6 × 5 mL) at 60 °C. After evaporation of the solvent from the collected ethereal phases, product

6 ea was purified by column chromatography on silica gel using as eluent pure hexane to 99 : 1 hexane-AcOEt (yield: 245 mg, 91%).

1-(3-(p-To~~lyl)~~benzo[b]thiophen-2-yl)ethan-1-ol (**6ea**). Yield: 64.0 mg, starting from 76.3 mg of **2e** (95%) (Table 4, entry 1). White solid, mp= 110–112 °C. IR (KBr): ν= 2214 (vw), 1597 (w), 1489 (m), 1462 (m), 1435 (m), 1254 (w), 1069 (m), 752 (s), 667 (m) cm^{-1}; ^{1}H NMR (CDCl , 500 MHz): δ= 7.86 (d, *J* = 7.4, 1H), 7.49 (d, *J* = 7.4), 7.35–7.25 (m, 6H), 5.24–5.18 (m 1H), 2.44 (s, 3 H), 2.00 (s, br, 1H), 1.59 (d, *J* = 6.4, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 146.0, 140.2, 138.3, 137.5, 133.5, 131.8, 129.8, 129.4, 124.5, 124.2, 123.2, 122.5, 65.0, 25.4, 21.3; GC-MS (EI, 70 eV): *m/z* = 250 [(M H_2O)$^+$, 59], 235 (100), 234 (69), 202 (12), 117 (16); HRMS-ESI (*m/z*): [(M-H_2O+H)$^+$] cald for ($C_{17}H_{15}S$)$^+$: 251.0889; found: 251.0898.

(3-(Thiophen-3-yl)benzo[b]thiophen-2-yl)methanol (**6gb**). Yield: 56.9 mg, starting from 72.8 mg of **2g** (92%) (Table 4, entry 2). White solid, mp= 75–78 °C. IR (KBr): ν = 1450 (m), 1366 (m), 1327 (w), 1219 (m), 1156 (s), 1134 (m), 949 (m), 895 (m), 849 (w), 756 (s) cm$^{—1}$; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.87–7.82 (m, 1H), 7.71–7.65 (m, 1H), 7.47 (dd, *J* = 4.9, 3.0, 1H), 7.41–7.37 (m, 1H), 7.37–7.32 (m, 2H), 7.25 (dd, *J* = 4.9, 1.3, 1H), 4.88 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 140.2, 139.8, 138.8, 134.4, 129.9, 128.8, 125.9, 124.8, 124.5, 124.3, 123.3, 122.6, 59.0; GC-MS (EI, 70 eV): *m/z* = 248 [(M+ 2)$^+$, 11], 246 (M$^+$, 100), 229 (25), 228 (23), 227 (23), 217 (44), 213 (28), 184 (40), 171 (16), 139 (10); HRMS-ESI (*m/z*): [(M-H_2O+H)$^+$] cald for ($C_{13}H_9S_2$)$^+$: 229.0140; found: 229.0149.

2-Butyl-3-(thiophen-3-yl)benzo[b]thiophene (**6ib**). Yield: 47.9 mg, starting from 79.3 mg of **2i** (70%) (Table 4, entry 3). Yellow solid, mp= 26–28 °C. IR (KBr): ν = 1458 (m), 1435 (m), 1381 (w), 1211 (w), 1173 (w), 1072 (m), 849 (m), 787 (m), 764 (s), 733 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.56–7.52 (m, 1H), 7.45 (dd, *J* = 4.9, 3.0, 1H), 7.31–7.23 (m, 3H), 7.17 (dd, *J* = 4.9, 1.0, 1H), 2.88 (t, *J* = 7.7, 2H), 1.71–1.63 (m, 2H), 1.41 1.31 (m, 2H), 0.87 (t, *J* = 7.4), 3H; ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 143.1, 140.4, 138.1, 135.4, 129.1, 128.3, 125.4, 124.1, 123.8, 123.7, 122.5, 122.1, 33.9, 28.8, 22.3, 13.8; GC-MS (EI, 70 eV): *m/z* = 274 [(M+ 2)$^+$, 8], 272 (M$^+$, 70), 229 (100), 185 (31), 184 (28), 115 (7); HRMS-ESI (*m/z*): [(M+H)$^+$] cald for ($C_{17}H_{17}S_2$)$^+$: 273.0766; found: 273.0771.

2-Butyl-3-phenylbenzo[b]thiophene (**6ic**). Yield: 45.6 mg, starting from 79.2 mg of **2i** (68%) (Table 4, entry 4). Yellow oil. IR (film): ν= 1605 (m), 1489 (m), 1435 (s), 1265 (m), 1196 (m), 1150 (m), 1072 (m), 1018 (m), 764 (s), 733 (m), 702 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.84–7.76 (m, 1H), 7.53–7.41 (m, 3H), 7.41–7.33 (m, 3H), 7.31 7.23 (m, 2H), 2.84 (t, J = 7.5, 2H), 1.66 (quintuplet, J = 7.5, 2H), 1.33 (hexuplet, J = 7.5, 2H), 0.86 (t, J = 7.5, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 142.6, 140.5, 138.3, 135.7, 133.5, 131.1, 128.5, 127.3, 124.1, 123.7, 122.6, 122.1, 33.9, 28.6, 22.3, 13.8; GC-MS (EI, 70 eV): m/z = 266 (M^+, 53), 223 (100), 221 (33), 178 (13); The spectroscopic data agreed with those reported.[15]

2,3-Diphenylbenzo[b]thiophene (**6mc**). Yield: 52.5 mg, starting from 84.2 mg of **2m** (73%) (Table 4, entry 5). White solid, mp= 112– 114 °C. IR (KBr): ν = 1597 (m), 1489 (w), 1435 (m), 1227 (w), 1072 (w), 1026 (w), 756 (s), 694 (m) cm^{-1}; ^{1}H NMR (CDCl , 500 MHz): δ= 7.85 (d, J = 7.8, 1H), 7.58 (d, J = 7.3, 1H), 7.41–7.28 (m, 9H), 7.25–1.17 (m, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 140.9, 139.6, 138.9, 135.5, 134.3, 133.3, 130.5, 129.6, 128.7, 128.4, 124.5, 124.4, 123.4, 122.1; GC-MS (EI, 70 eV): m/z = 286 (M^+, 100), 271 (16), 252 (15), 142 (8). The spectroscopic data agreed with those reported.[8c]

3-Phenyl-2-(p-tolyl)benzo[b]thiophene (**6nc**). Yield: 52.1 mg, starting from 87.9 mg of **2n** (69%) (Table 4, entry 6). White solid, mp= 167– 168 °C. IR (KBr): ν = 1597 (w), 1481 (w), 1435 (m), 1242 (m), 1157 (m), 1026 (w), 872 (w), 748 (s), 694 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.83 (d, J = 6.9, 1H), 7.56 (d, J = 8.0, 1H), 7.42–7.25 (m, 7H), 7.22– 7.17 (m, 2H), 7.05–7.00 (m, 2H), 2.29 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 141.0, 139.8, 138.8, 137.6, 135.7, 132.8, 131.4, 130.5, 129.5, 129.1, 128.6, 127.3, 129.4, 123.2, 122.0, 21.2; GC-MS (EI, 70 eV): m/z = 300 (M^+, 100), 285 (37), 284 (41), 252 (11), 142 (14). The spectroscopic data agreed with those reported.[8q]

2,3-Di(thiophen-3-yl)benzo[b]thiophene (**6pb**). Yield: 60.0 mg, start- ing from 85.8 mg of **2p** (80%) (Table 4, entry 7). White solid, mp= 123–127 °C. IR (KBr): ν= 1597 (m), 1489 (w), 1435 (m), 1227 (w), 1072 (w), 1026 (w), 756 (s), 694 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ= 7.86–7.77 (m, 1H), 7.61–7.52 (m, 1H), 7.46–7.39 (m, 1H), 7.37–7.26 (m, 3H), 7.25–7.15 (m, 2H), 7.06 (d, J = 4.7, 1H), 6.97–6.90 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 141.0, 138.1, 135.5, 134.84, 134.81, 129.2, 127.8, 127.7, 125.8, 125.5, 124.62, 124.55, 124.4, 123.5, 123.2, 122.0; GC-MS (EI, 70 eV): m/z = 300 [(M +

2)$^+$, 19], 298 (M$^+$, 100), 297 (65), 264 (41), 253 (43), 221 (16), 149 (9), 132 (18); HRMS-ESI (*m/z*): [(M+ H)$^+$] cald for ($C_{16}H_{11}S_2$)$^+$: 299.0017; found: 299.0045.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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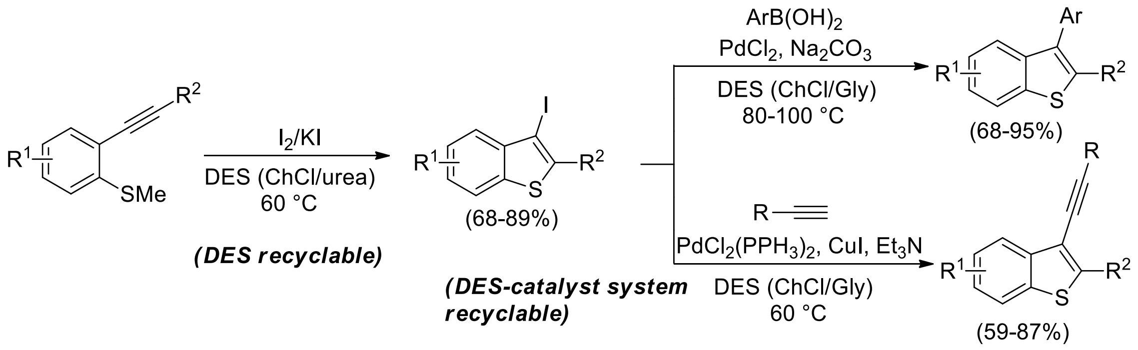

The iodocyclization of 2-methylthiophenylacetylenes can be conveniently carried out in the ChCl/urea (1/2, mol/mol) deep eutectic solvent as recyclable and more sustainable solvent with respect to the classical organic solvents employed so far. The obtained 3-iodobenzothiophenes have been successfully used as substrates in cross-coupling reactions in the ChCl/Gly DES with the possibility to easily recycle the solvent-catalyst system.

Conclusions

Research confirms that DESs are a viable alternative to traditional solvents in the field of cultural heritage and chemistry. Beginning with a thorough investigation of their chemical and physical properties, it shows significant advantages in their use, meeting the requirements to be called "green".

In the field of cultural heritage, DESs are first evaluated in situ as biocidal agents for the removal of biological patina from an ancient Roman mosaic. Although the biocidal efficacy of DES is lower than that of conventional biocides, such as Preventol RI50, the benefits and safety for operators and the environment offered by DES make it a new sustainable choice. Following this, the research focuses on the study of hydrophobic DESs to be used in the removal of aged non-polar coatings commonly used as sacrificial layers to protect stone and metal artifacts of cultural interest. The study is conducted in the laboratory, evaluating the cleaning action of DESs in the removal of beeswax and two microcrystalline waxes (R21 and Reinassance) The effectiveness in removing aged wax coatings is given by the structural affinity between a given DES and a particular type of wax.

Preliminary results obtained from this initial stage of research are very promising in terms of their possible use as solvents in the cleaning and conservation of artworks. They have all the necessary characteristics to be able to replace classical toxic and flammable organic solvents. Their low volatility makes it possible to use less solvent, as DES persists on the surface for a longer period without evaporating, thus reducing the risk of damage to the environment and the operator. In addition, their liquid appearance at room temperature eliminates the need to add diluent solvents for ease of use, and their transparent properties make them suitable for use in the cleaning and preservation of works of art.

In the field of organic synthesis, DESs are effective as unconventional solvents for iodocyclization, Sonogashira coupling, and Suzuki coupling reactions. The methodology developed for the synthesis of 3-iodobenzothiophene products through iodocyclization reactions conducted in DES ChCl/U yields desired products in good yields, confirming the generality and recyclability of this process. Subsequently, the iodocyclization products are subjected to Sonogashira and Suzuki coupling reactions in DES ChCl/Gly. The recyclability of the solvent-catalyst system emerges as an additional advantage, confirming the suitability of DESs as green solvents for synthesizing molecules in an environmentally sustainable method. These promising results not only underscore new strategies for the synthesis of variously functionalized compounds but also enable the development of innovative molecules that may be of interest in various application areas.

Future Perspectives

The results obtained so far in both the fields of restoration and organic synthesis are promising, providing a solid foundation for the continued experimentation with DESs.

An innovative aspect of future research will be the development of advanced physical-mathematical models for the quantitative study of the permeation and diffusion capabilities of DESs, deepening the understanding of solvent-material interactions. Special attention will be paid to the design and creation of membranes incorporating DESs into polymer networks. These materials will be adapted for cleaning and conservation of artifacts.

In the field of organic synthesis, the next goal will be to use simple and readily available substrates to conduct organic chemistry reactions using DESs as safe and recyclable solvents. This strategy aims to generate new organic molecules that can serve as precursors for more complex compounds. Applications of these new molecules will extend to areas as diverse as medicine, agro-chemistry and materials science.

On an international scale, the adoption of DESs represents a crucial opportunity to promote new practices in both cultural heritage conservation and organic synthesis. Collaboration with international institutions and research groups will be critical in expanding the scope of this research. Given the early stage of the study, it is essential to conduct further in-depth investigations that consider all aspects of DES and truly assess its benefits and potential applications.

This initiative not only aims to validate the effectiveness of DESs as solvents for cultural heritage conservation, but also aims to promote the adoption of recyclable solvents in chemical synthesis.

This process will consolidate DESs as new environmentally friendly solvents in various scientific and industrial applications. These efforts will contribute significantly to a sustainable future in different fields through innovative and eco-friendly solutions.