Epoxy–amine oligomers from terpenes with applications in synergistic antifungal treatments

Dara M. O’Brien, Cindy Vallieres, Cameron Alexander, Steven M. Howdle, Robert A. Stockman* and Simon V. Avery*

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| First (given) and middle name(s) | Last (family) name(s) | ResearcherID | ORCID ID         |
|----------------------------------|-----------------------|--------------|-----------------|
| Dara M.                          | O’Brien               |              | 0000-0002-5916-8364 |
| Cindy                            | Vallieres             |              |                 |
| Cameron                          | Alexander             | N-7729-2014  | 0000-0001-8337-1875 |
| Steven M.                        | Howdle                | A-2954-2010  | 0000-0001-5901-8342 |
| Robert A.                        | Stockman              | G-7003-2011  | 0000-0002-7915-340X |
| Simon V.                         | Avery                 |              |                 |
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Epoxy–amine oligomers from terpenes with applications in synergistic antifungal treatments†

Dara M. O’Brien, Cindy Vallieres, Cameron Alexander, Steven M. Howdle, Robert A. Stockman and Simon V. Avery*

A bis-epoxide monomer was synthesised in two steps from (R)-carvone, a terpenoid renewable feedstock derived from spearmint oil, and used to prepare β-aminoalcohol oligomers in polyaddition reactions with bis-amines without requiring solvent or catalyst. A sub-set of the resultant materials were readily water soluble and were investigated for antifungal activity in combination with the fungicide iodopropynyl-butylcarbamate (IPBC) or the antifungal drug amphotericin B. The oligo-(β-aminoalcohols) alone were inactive against Trichoderma virids and Candida albicans but in combination with IPBC and amphotericin B demonstrated synergistic growth-inhibition of both fungi. Quantitative analysis showed that the presence of the terpene-based oligomers decreased the minimum inhibitory concentration (MIC) of IPBC by up to 64-fold and of amphotericin B by 8-fold. The efficacy of the combined formulation was further demonstrated with agar disk diffusion assays, which revealed that IPBC and amphotericin B reduced the growth of the fungi, as shown by zones of inhibition, to a greater extent when in the presence of the oligo-(β-aminoalcohols). These data suggest potential future use of these renewable feedstock derived oligomers in antifungal material and related biomedical applications.

The importance of epoxy resins can be gauged by their global market size, which in 2015, was ~$8 billion.9 These materials account for a sizable part of the polymer industry, but this is becoming increasingly problematic as almost all epoxy polymers are petroleum-derived. Sustainability,10 along with environmental11 and economic12 issues surrounding the use of crude oil demand that new, renewable feedstocks are found for polymer materials.

Research into renewable epoxy resin materials has examined both the epoxide monomers as well as their curing agents.13 Investigations into alternative, bio-based monomers have focused on synthesising bis-epoxides with similar structures and properties to those of the most common counterparts derived from crude oil.14 Xu et al. showed that certain cured resins of de-polymerised Kraft and organosolv lignin showed promise as a substitute for petroleum-based epoxy resins.15 Similarly, Caillol et al. developed a novel epoxy-monomer from eugenol, a monolignol primarily found in cloves. When cured with aliphatic and aromatic amines, the resins were found to form thermosets with high glass transition temperatures and good thermomechanical properties.10 These are only two examples as there is now a rapidly developing literature on the synthesis of renewable epoxy resins from bio-based sources such as lignin, rosin, tannins and sugars.16

Of the molecular biomass available, terpenes have generated substantial interest as feedstocks for renewable polymers, with numerous reviews on the subject.2,17,18 Terpenes are formed in

Introduction

Since their discovery in the early twentieth century, synthetic polymers have been rapidly incorporated into almost every aspect of modern life.1,2 In addition to large-scale uses in clothing and packaging, synthetic polymers are increasingly finding application in fields as diverse as electronics, high performance structures and in medicine.1–4 Epoxide-based polymers, first commercialised in the 1940s,5 have been particularly well-developed as a result of their excellent mechanical strength, electrical insulation and thermal resistance.6 The most common industrial use of epoxide-based polymers (‘epoxy resins’) is in thermosetting materials and protective coatings.5 However, more recently, aliphatic epoxide systems have been employed to produce materials with potential biomedical applications. For example, Hamid et al. used epoxy-amine polymerisations to form hydrogels as tissue engineering supports and drug delivery systems,7 while González García et al. formed epoxide-derived-networks with cycloaliphatic amines which exhibited cytocompatibility properties suitable for use in cardiovascular applications.8

9 School of Chemistry, University Park University of Nottingham, NG7 2RD, UK
10 School of Life Sciences, University Park University of Nottingham, NG7 2RD, UK
11 School of Pharmacy, University Park University of Nottingham, NG7 2RD, UK
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a biosynthetic pathway common to a variety of trees, plants, fungi and insects,\textsuperscript{19} are naturally abundant, and do not compete directly with food sources, making them attractive renewable building blocks for a variety of applications.\textsuperscript{20} Terpenoids are similar isoprene-based compounds that have incorporated oxygen into their structures.\textsuperscript{21}

Despite their abundance, monoterpenes have not been explored to date in the generation of new monomers for epoxy-resin chemistry. In this paper, we describe the synthesis of a terpene-based epoxy monomer from (R)-carvone, a terpenoid found in caraway and spearmint oils in concentrations of 60\% and 70–80\%, respectively.\textsuperscript{22} While it can be extracted from nature, (R)-carvone is also synthesised from limonene,\textsuperscript{22} a terpene produced as a waste product of the citrus industry; the (R)-enantiomer alone is produced on a scale of 70 kilotons per annum.\textsuperscript{17} This, along with the functional groups of the molecule, make (R)-carvone an attractive starting material for the synthesis of a bis-epoxide monomer. We describe how an epoxide monomer can be synthesised from (R)-carvone using two parallel routes, and compare the conventional epoxidising agent meta-chloroperbenzoic acid (mCPBA), with that of in situ generated dimethyl dioxirane (DMDO) as electrophilic oxidising agents. For these reactions, we formed DMDO from the reaction of Oxone\textsuperscript{26} in acetone, using conditions adapted from those in the literature.\textsuperscript{25} We subsequently employed hydrogen peroxide in a second step as a nucleophile epoxidising agent. These methods allowed for the synthesis of the bis-epoxide monomer from a sustainable feedstock, using low hazard oxidising agents and avoiding the use of chlorinated solvents.

We subsequently polymerised the epoxy-based monomers with commercially available amines via simple step-growth, polyaddition reactions.\textsuperscript{7} The reaction did not require additional components such as initiators or coupling agents, making it appealing in terms of sustainability and any potential biomedical applications of the resulting materials.\textsuperscript{24} We further demonstrated that the carvone-based bis-epoxide monomer could undergo curing with a commercially available, di-secondary diamine, also employing mild reaction conditions with gentle heating.

With the overall aim of this study being to construct new, renewable materials with suitable properties for use in biomedical or anti-biofouling applications, fungal inhibition by the resulting oligomers was then investigated. The development of new anti-fungal agents has encountered a number of challenges, including those of antifungal resistance.\textsuperscript{25–27} Synergistic combinations of agents may offer a pragmatic approach to this problem, as potent new formulations can be developed without the need for specific new agents, and these approaches may also be facilitated by repurposing known antifungals.\textsuperscript{26,27} Additionally, synergistic combinations enable lower amounts of active agents to be used.\textsuperscript{26} An anticipated membrane action of the oligomers studied here supported the idea that it could act synergistically with other inhibitory agents (discussed later). We thus sought to investigate the use of our epoxy-amine oligomers in synergistic antifungal treatments with the known fungicide iodopropynyl butylcarbamate (IPBC) and the widely-used antifungal amphotericin B, against the environmental fungus Trichoderma virens (a common contaminant) and the major human pathogen Candida albicans, respectively.

**Experimental section**

**Materials**

Commercially available chemical reagents were purchased from Alfa Aesar, Acros Organics, Merck, Sigma Aldrich or Fischer Scientific UK and used as received unless otherwise stated. Solvents were purchased from Fischer Scientific UK and used without further purification unless otherwise stated. Water was deionised before use. Brine is a saturated aqueous solution of sodium chloride. Solvent evaporation was performed using a rotary evaporator under reduced pressure. Reactions were monitored by TLC (Thin Layer Chromatography) carried out on aluminium-backed plates coated with Merck Kieselgel 60 F254, and visualised using KMnO$_4$ stain and gentle heating. Amphotericin B from Streptomyces sp. along with fungicide 3-iodo-2-propynyl N-butylcarbamate, were purchased from Sigma Aldrich. All compounds used in the anti-fungal assays were prepared in DMSO, and added to growth media to give the specified final concentrations.

**General methods and instrumentation**

**Nuclear magnetic resonance.** Bruker AV400 and AV3400 NMR spectrometers operating at 400 MHz ($^1$H) and 101 MHz ($^{13}$C) at ambient temperature were used to perform nuclear magnetic resonance (NMR) analysis in deuterated solvents. Chemical shifts were assigned in parts per million (ppm). $^1$H NMR chemical shifts ($\delta_{\text{H}}$) are reported with the shift of CHCl$_3$ ($\delta = 7.26$ ppm) as the internal standard when CDCl$_3$ was used. $^{13}$C chemical shifts ($\delta_{\text{C}}$) are reported using the central line of CHC1$_3$ ($\delta = 77.0$ ppm) as the internal standard. All spectra were obtained at ambient temperature ($22 \pm 1^\circ$C). MestReNova 6.0.2 copyright 2009 (Mestrelab Research S. L.) was used for analysing the spectra.

**Mass spectrometry.** High Resolution Mass Spectrometry (HRMS) was conducted using a Bruker MicroTOF spectrometer operating in electrospray ionisation (ESI) mode.

**Fourier-transform infra-red spectroscopy.** FTIR spectroscopy was performed in the range of 4000–650 cm$^{-1}$. This was carried out using a Bruker Tensor 27 FT-IR spectrophotometer using an ATR attachment. Spectra were analysed using MicroLab software.

**Gel permeation chromatography (GPC).** Gel permeation chromatography (GPC) was used for determination of number average molecular weight ($M_n$), weight average molecular weight ($M_w$), peak molecular weight ($M_p$) and molecular weight distribution (polydispersity, $D$, $M_w/M_n$). The analysis was performed using an Agilent 1260 Infinity Series HPLC (Agilent Technologies, USA) fitted with a differential refractive index detector (DRI). THF (HPLC grade, Fisher Scientific) was used as eluent at room temperature using two Agilent PL-gel mixed-E
columns in series at a flow rate of 1 mL min⁻¹. A calibration curve was made using polycaprolactone standards.

**Synthesis of α-epoxy-ketone, 1**

To a solution of (R)-carvone (10.4 mL, 66.6 mmol) in MeOH (100 mL) was added 1 M NaOH solution (20 mL, 20.0 mmol) and H₂O₂ (30% w/w, 8.4 mL, 80.0 mmol). The mixture was stirred for 24 hours before quenching excess peroxides with sat. aq. Na₂S₂O₃ solution (100 mL). The aqueous layer was then extracted with CH₂Cl₂ (100 mL) and the combined organic extracts were washed with brine (2 × 100 mL) and dried over Na₂SO₄. Volatiles were removed under reduced pressure and the product was then concentrated in vacuo to afford the title compound as a yellow oil (9.37 g; 85%) as a mixture of diastereomers (9:1), only peaks for the major diastereomer (1a) are reported.

**Synthesis of bis-epoxide, 2, using mCPBA**

To a solution of 1 (9.37 g, 56.47 mmol) in CH₂Cl₂ (100 mL) at 0 °C was added meta-chloroperbenzoic acid (mCPBA) (70% w/w, 16.7 g, 67.8 mmol) slowly over 10 minutes. The solution was stirred for 16 hours at room temperature before excess peroxides were quenched with sat. aq. Na₂S₂O₃ solution (100 mL). The aqueous layer was then extracted with CH₂Cl₂ (100 mL) and washed with NaHCO₃ (3 × 150 mL), and brine (100 mL) before drying over MgSO₄. The solvent was removed under reduced pressure to yield title compound as a mixture of diastereomers as a yellow oil (yield: 8.82 g, 90%, dr = 9:9:1:1, i.e. (1R,4R,6R,7S):(1R,4R,6R,7R):(1S,4R,6S,7S):(1S,4R,6S,7R)). Only peaks for the two major diastereomers (2a and 2b) are reported (see ESI† for further details).

**Fungal growth inhibition assays**

For growth assays with *Candida albicans* SC5314, a standard laboratory strain, single colonies from YPD agar plates were used to inoculate YPD broth cultures [2% peptone (Oxoid, Basingstoke, UK), 1% yeast extract (Oxoid), 2% D-glucose] in Erlenmeyer flasks and incubated overnight at 37 °C with orbital shaking at 120 rpm. Overnight cultures were diluted to OD₆₀₀ ~ 0.5 and cultured for a further 4 hours in fresh medium. The 4 hour mid/late exponential cultures were diluted to OD₆₀₀ ~ 0.01 and 100 μL aliquots transferred to 96-well microtiter plates (Greiner Bio-one; Stonehouse, UK), with drugs and oligomer added to final concentrations as specified in the Results section. Plates were incubated at 37 °C with shaking in a BioTek Powerwave XS microplate spectrophotometer and OD₆₀₀ was recorded every 30 min. For growth assays with *Trichoderma virens* CBS 430.54, spores were harvested from 7 d PDA (Oxoid) plates and inoculated to PDB (Sigma-Aldrich) broth to a concentration of 15 000 spores per mL. Aliquots (100 μL) of the diluted culture plus any chemical supplements, as specified in the Results section, were transferred to 96-well plates and cultured statically for up to 65 hours at 30 °C in a BioTek Powerwave XS microplate spectrophotometer. For assaying antifungal combinations, concentrations of agents used were those determined from preliminary assays to be just subinhibitory or slightly inhibitory when supplied individually.

**Checkerboard assays**

General culturing and preparation for yeast ‘checkerboard’ assays was according to EUCAST guidelines.²⁸ Briefly, yeast cells from single colonies were inoculated from 2-d PDA plates to RPMI 1640 medium + 2% glucose and cell concentration adjusted to a final inoculum of 10⁵ cells per mL. All culturing for checkerboard assays with *T. virens* was as described above. Culture aliquots (100 μL) were transferred to 96-well microtiter plates with chemicals added at the concentrations specified in the checkerboard figures (below). The inoculated plates were incubated statically for 48 hours at 30 °C (*T. virens*) or 24 hours at 37 °C (*C. albicans*). OD₆₀₀ was then measured with a BioTek EL800 microplate spectrophotometer. Fractional Inhibitory Concentration (FIC) as an indicator of synergy was calculated as described.²⁹

**Disk diffusion assays**

PDA and YPD medium were inoculated with 10⁶ spores per mL (*T. virens*) from 7 d-PDA plates and 10⁷ cells per mL from exponential growth-YPD broth cultures (*C. albicans*), respectively. Sterile Whatman paper filter disks loaded with 10 μL inhibitor(s) were laid on the plates and incubated at 30 °C for 48 hours (*T. virens*) or at 37 °C for 24 hours (*C. albicans*).

**Results and discussion**

**Synthesis of bis-epoxide monomer, 2**

Two parallel routes towards the synthesis of a carvone-based bis-epoxide monomer were designed, through the sequential
epoxidations of the double-bonds in the molecule (Scheme 1). These double bonds are electronically distinct from one another,\(^{30}\) which gives rise to the regioselective epoxidation at either the endo- or exocyclic positions.\(^{30}\) The endocyclic double bond is electron deficient due to its conjugation with the ketone, so epoxidation at this position was achieved using alkaline hydrogen peroxide, which reacts as a nucleophilic oxidising agent. The \(\alpha\)-epoxy ketone (1) was obtained in 90% yield, in a diastereomeric ratio of 9 : 1 for the epoxide in the (R,R):(S,S) positions, i.e. 1a and 1b, (Scheme 1), respectively. The diastereometric ratio was determined using \(^1\)H NMR spectroscopy, according to the integration of the resonances corresponding to the methyl group in the \(\alpha\)-position to the carbonyl moiety. The peak positions for these shifts are found at 1.70 ppm and 1.69 ppm for 1a and 1b, respectively. The stereochemical selectivity displayed in this reaction is thought to be a result of the formation of the more favourable chair-like transition state via axial attack of the hydroperoxide anion. In this reaction, the exocyclic alkene remained completely intact.\(^{31}\)

The mixture of \(\alpha\)-epoxy ketones 1a and 1b was then treated with \(m\)CPBA to oxidise the electron-rich, exocyclic alkene. No stereoselectivity was observed in this reaction, resulting in a mixture of bis-epoxide species 2a to 2d in a 90% yield (Scheme 1, route 1). By this route, the monomer was thus synthesised in a two-step process with an overall yield of 81%, in a diastereomeric ratio of 9 : 9 : 1 : 1. It was found that epoxidising the double bonds in the reverse order, i.e. the exocyclic double bond first with \(m\)CPBA, followed by the endocyclic one with \(H_2O_2\), brought about the same results in similar yields and diastereomeric ratios; we did not find either sequence to have a distinct advantage over the other. Attempts to use \(m\)CPBA to bring about the epoxidation of both double bonds in one step were not successful.

The use of \(m\)CPBA is not atom economic and produces a biproduct, chloroperbenzoic acid, as stoichiometric waste. The reaction is also very exothermic, a particular issue when working at a large scale, and makes use of dichloromethane, a suspected carcinogen. To avoid the use of this reagent, an alternative synthesis was designed, using dimethyl dioxirane as the oxidant. This can be made \textit{in situ} from the reaction of acetone and Oxone\(^{36}\), the commercial name for potassium peroxymonosulfate, an inexpensive, stable salt.\(^{23}\) This method avoids the use of metal catalysts, is cheap, and the organic product can be easily isolated without the use of chlorinated solvents, or the need for further purification.\(^{23}\)

When the mixture of \(\alpha\)-epoxy ketones 1a and 1b was treated with Oxone\(^{36}\) in acetone, the bis-epoxide monomer species 2a to 2d were produced in a yield of 83%, resulting in an overall yield over the two steps of 75% (Scheme 1, route 2). This is comparable to the \(m\)CPBA route, and as such the Oxone\(^{36}\) route can be considered the most advantageous as it significantly improves the sustainability of the monomer synthesis. As with \(m\)CPBA, attempts to synthesise the bis-epoxide monomer in one step using Oxone\(^{36}\) were not successful.

We were not concerned with isolating the monomer as a pure diastereomer and did not attempt to separate or isolate the mixture of diastereomers of either 1 or 2. For simplicity, the stereochemistry is not represented in this article.

**Synthesis of epoxy–amine oligomer 4, with bis-epoxide 2 and secondary diamine 3**

Typically, the reaction of a bis-epoxide and a diamine curing agent is known to proceed at ambient temperature\(^{32}\) and in the absence of coupling agents or initiators.\(^7\) Curing is usually performed with primary or secondary di- or poly-amines.\(^{33}\) These may be aliphatic, cycloaliphatic or aromatic.\(^6\) Following the synthesis of the terpene-based bis-epoxide monomer 2, its step-growth polymerisation was investigated by reaction with the commercially available, aliphatic, di-secondary diamine, \(N^3,N^6\)-dimethylhexane-1,6-diamine, 3 (Scheme 2). This diamine was specifically chosen as it features a relatively long, flexible hexyl linker, which was anticipated would minimise the possibility of intra-molecular cyclisation. Initially, the monomers were mixed together in a 1 : 1 ratio, in the absence of solvent or catalyst, and heated to temperatures ranging from 0 °C to 110 °C, for 5 hours using an oil bath. NMR analyses indicated that samples conducted from 0 °C to 50 °C returned the two monomers, without any reaction. However, for samples conducted from 70 °C to 110 °C, it was evident by NMR and IR analysis that the reaction had proceeded, and no further trace of the bis-epoxide monomer could be detected by \(^1\)H NMR (Fig. 1A). GPC analysis indicated the formation of populations
of short-chain oligomers for samples conducted \( \geq 70 \, ^\circ C \) (Fig. 1B).

A range of experimental conditions were tested in attempts to control the molar masses of the samples, including temperatures of 22 \(^\circ\)C, 50 \(^\circ\)C, 70 \(^\circ\)C and 90 \(^\circ\)C, and reaction times of 24 hours, 4/5 days and 7 days. By GPC analysis, it was evident that curing times of 24 hours led to the formation of small populations of oligomers with increased molar mass, relative to those conducted at the same temperature for only 5 hours. Longer times of up to one week were required to increase sufficiently the mass fractions of these populations (Fig. 1C).

In each case, longer reaction times combined with higher temperatures increased the viscosity of the samples, to the point at which the vials could be inverted without any movement of the sample. These samples were also less soluble in a variety of solvents than their counterparts conducted over only 5 hours. It is unlikely that the polymer chains cross-linked during the curing process, as, when considering the base-catalysed mechanism for ring-opening of epoxides, each secondary amine becomes tertiary after just a single addition to an epoxide. Additionally, the hydroxyl groups formed are predicted to be tertiary, and therefore unlikely to undergo nucleophilic attack. While the alternative, acid-catalysed mechanism would produce a primary and secondary alcohol which would be able to crosslink, we anticipate that this product was unlikely to form in these conditions, due to the presence of the basic di-amine monomer. As such, cross-linking via the predicted tertiary hydroxyl groups was not considered further.

As the ratio of epoxide to amine was kept strictly at 1:1, minimal cross-linking would occur between the end-groups of the oligomers, and so the difference in viscosity were most likely caused by increased proportions of higher mass-fractions of the populations of oligomers present, with those with higher amounts of higher-mass populations inducing more viscous properties to the overall sample. NMR spectra for the samples were consistent, but were complex, and were not diagnostic in

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**Fig. 1** \(^1\)H NMR spectrum showing complete conversion of bis-epoxide monomer to cured oligomers (A). GPC chromatogram indicating the presence of short-chain oligomers in samples conducted \( \geq 70 \, ^\circ C \) (B), and GPC chromatogram indicating the effect of time on the mass-fraction of short-chain oligomers with higher \( M_n \) (C).
the fungus. However, amphotericin B is also nephrotoxic, leakage of cellular components, ultimately resulting in death of the formation of pores in the cell membrane, which allows the need for aqueous solubility in our formulations, we therefore did not investigate further the potential anti-fungal activity of these oligomers.

Growth inhibition and checkerboard assays

Synergistic activity of the oligomers of 4 with the antifungal drug amphotericin B and the fungicide iodopropynyl butylcarbamate (IPBC) were investigated against Candida albicans and Trichoderma virens, respectively (Fig. 2).

Amphotericin B is a polyene antifungal which has proven activity against Candida species. It binds to sterols leading to the formation of pores in the cell membrane, which allows leakage of cellular components, ultimately resulting in death of the fungus. However, amphotericin B is also nephrotoxic, which limits its use to doses of between 3–5 mg kg

Fig. 2 The structures of iodopropynyl butylcarbamate (IPBC) and amphotericin B.
disks were soaked with the inhibitors and laid on agar plates inoculated with *C. albicans*. The susceptibility of the fungus after 24 hours to the combination of 4 with amphotericin B was evident by zones of inhibition which were greater with the agents combined than by a simple product of their individual effects at the same doses.

Unlike *C. albicans*, *Trichoderma virens* is not pathogenic to humans. However, fungicidal treatments against *Trichoderma virens* are valuable for materials applications, for example, in protecting paints, sealants or coatings from fungal colonisation. The synergistic activities of the oligomers of 4 against *T. virens* were investigated together with IPBC, which is widely used in the cosmetics industry and as a preservative in painting, textiles and adhesives.\(^{37,38}\) However, its use has also caused allergic reactions and thus there is a need to reduce the levels of IBPC incorporation in formulations for more widespread applications.\(^{37}\)

A growth inhibition assay was conducted with compounds provided at sub-inhibitory concentrations, as done above with *C. albicans*. When combined in this way, 4 and IPBC produced a complete inhibition of fungal growth (Fig. 4A). Checkerboard analyses were again used and these indicated (Fig. 4B) synergistic antifungal activity between the oligomers of 4 and IPBC.

The presence of just 250 µg mL\(^{-1}\) of 4 was found to decrease the MIC of IPBC by up to 64-fold, from 1600 ng mL\(^{-1}\) to 25 ng mL\(^{-1}\). The FIC for the combination was found to be 0.37, indicating a synergistic relationship.\(^{36}\) Synergy was also observed for the two monomers (FIC = 0.31 and 0.38 for 2 and 3, respectively). However, MICs of 4.5 mg mL\(^{-1}\) (2) and 1.8 mg mL\(^{-1}\) (3) were found for these small molecules, highlighting the advantage of the oligomeric system which required much less material as the MIC of 4 was only 0.5 mg mL\(^{-1}\). The evidence for a synergistic relationship in activity for IBPC and the oligo-β-aminoalcohol 4 was supported by means of a disk diffusion assay: sterile filter paper disks were soaked with the inhibitors and laid on agar plates inoculated with *T. virens*. Two concentrations of IPBC, 25 µg mL\(^{-1}\) and 50 µg mL\(^{-1}\), were inoculated alongside 0 mg mL\(^{-1}\) and 100 mg mL\(^{-1}\) of the oligomers. The susceptibility of *T. virens* to the combination of 4 with IPBC was visually evident by zones of inhibition, which could be observed after 48 hours (Fig. 4C). The anti-fungal relationship was, as expected, more evident with the higher doses of both the fungicide and 4.

The potential mechanisms underlying the synergistic activity of the antifungal agents and oligo-β-aminoalcohol 4 are likely to involve interactions of the compounds with fungal membranes. Oligomer 4 contains tertiary amine groups, H-bonding hydroxyls and also a degree of lipophilicity from the substituted hexane repeat units. As a consequence, the oligomers were probably partially charged at the pH values of the assays and thus amphiphilic. It has been well-established that a degree of membrane activity from amphiphilic components can enhance the efficacy of anti-fungal agents,\(^{39}\) and it has also been very recently shown that polycations such as poly(Lysine) and DEAE dextran hydrochloride have membrane-disrupting effects on *C. albicans*.\(^{40}\) Since it has also been established that polycations induce leakage of cell contents in yeasts,\(^{41}\) we think it possible that the synergy we observed with 4 and IBPC and Amp B arose due to a combination of membrane disruption and conventional antifungal activity. The cell walls of fungi contain multiple glycoproteins and polysaccharides, but vary not only with the specific fungal strain but also with the environment in which the fungi inhabit. It is therefore challenging to ascribe specific anti-fungal membrane effects to components in a formulation without a systematic evaluation of each individual component under well-defined conditions. However, we are currently preparing further derivatives of these oligomers with a view to testing the membrane activity hypothesis and will report these data in a future manuscript.

**Conclusions**

In this study we have successfully synthesised a bis-epoxide monomer from a renewable terpene feedstock, in high yield over two steps. We have shown that this can be achieved using inexpensive chemistry and under mild conditions, and have eliminated the need for mCPBA. This monomer was then used in a step-growth polyaddition mechanism with a commercially available, aliphatic, secondary diamine using well-established epoxy–amine chemistry. In the absence of catalysts or solvents, this reaction was found to produce populations of low molecular weight oligomers. While optimisation of this polymerisation might be useful to investigate a higher conversion
of these oligomers to longer chain polymers, the shorter-chain materials were found to have suitable properties for use in antifungal applications: the oligomer 4 was found to act in synergy with known fungicide IPBC and the antifungal drug amphotericin B against *Trichoderma virens* and *Candida albicans*, respectively. This was demonstrated using growth inhibition assays, checkerboard assays, and disk diffusion assays. In the case of both fungi, the addition of the oligomers of 4 was found to reduce the MIC of the respective antifungal agent.

These can be considered positive preliminary results for the future use of these oligomers in antifungal material and biomaterial applications.

**Data access statement**

All raw data created during this research are openly available from the corresponding authors (robert.stockman@nottingham.ac.uk and simon.avery@nottingham.ac.uk) and at the University of Nottingham Research Data Management Repository (https://rdmc.nottingham.ac.uk/) and all analysed data supporting this study are provided in the ESI† accompanying this paper.

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**Conflicts of interest**

The authors declare no conflict of interest.

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