JACS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

pubs.acs.org/JACS

Article

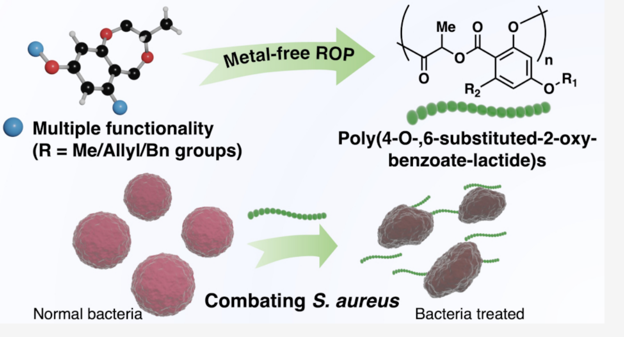
Biosourced Functional Hydroxybenzoate-*co*-Lactide Polymers with Antimicrobial Activity

Pedro Salas-Ambrosio, Shelby Vexler,[⊥] Rajalakshmi P Sivasankaran,[⊥] Niko Vlahakis, Ryan S. Lai, Colin Johnson, Sophia I. Baas-Maynard, Daniel S. Min, Haillie Lower, Abigail G. Doyle, Yi Tang, Jose A. Rodriguez, Irene A. Chen,* Javier Read de Alaniz,* and Heather D. Maynard*

Cite This: https://doi.org/10.1021/jacs.5c04624

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Antimicrobial resistance is an urgent global health challenge, and compounds that address this issue have attracted significant attention. In particular, bioderived molecules that possess natural antimicrobial properties can be useful to prepare active macromolecules that are degradable. In this work, a 4-(methyl/allyl/benzyl)oxy-6-(H/alkyl)-2-oxy-benzoate-*co*-lactide-based polymer library was designed and studied for antimicrobial activity. The monomer precursors were heterologously produced and purified from an engineered fungal host, chemically modified with 4-(methyl/allyl/benzyl)oxy substituents, and ring-closed to form the 3-methyl-5*H*-benzo[*e*][1,4]dioxepine-2,5(3*H*)-diones. The polymers were synthesized by ring-opening polymerization using a 3-O urea/1-methyl-2,3,4,6,7,8-hexahydro-1H-pyrimido[1,2-*a*]pyrimidine catalytic system and 3-methyl-butan-1-ol as the initiator. Polymers at different degrees of polymerization were prepared by varying the [monomer]/[initiator] (M/I = 5−30) and tested for activity against the pathogen *Staphylococcus aureus*. Polymers were identified that were antimicrobial and disrupted biofilms while maintaining good in vitro biocompatibility. The degradability of the polymers was confirmed. Overall, these results demonstrate the power of utilizing a combination of synthetic biology and chemistry to produce functional and degradable polymers that are potent inhibitors of the Gram-positive bacterium *S. aureus*, with potential applications in medicine.

■ INTRODUCTION

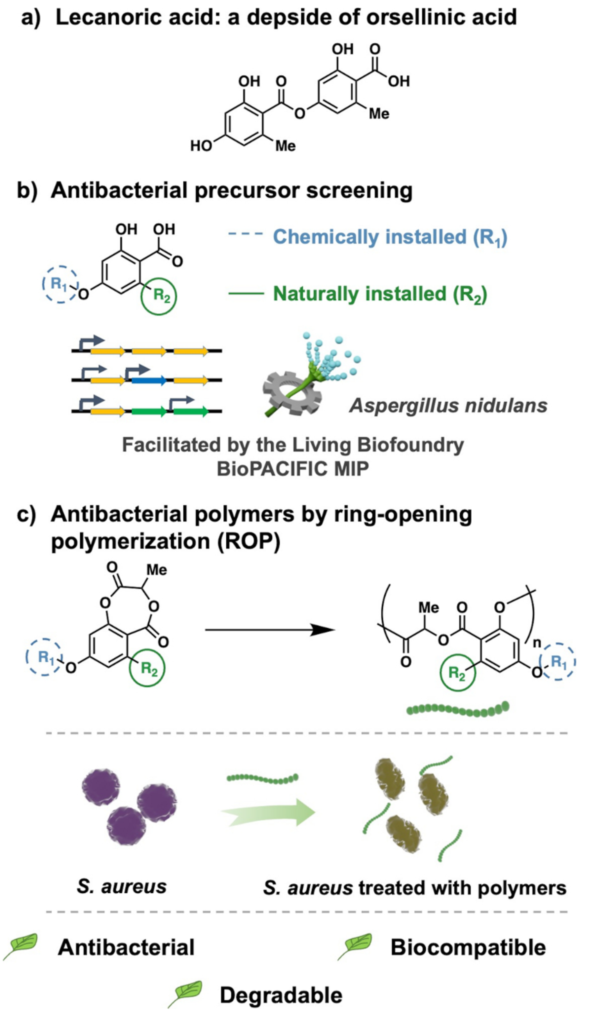
Antimicrobial resistance (AMR) currently threatens global healthcare, and finding new treatments is an urgent challenge.[1,2] Since the discovery by Fleming of penicillin that was isolated from fungi,[3] multiple antimicrobials have been discovered as natural products such as antimicrobial peptides (AMPs).[4,5] Currently, synthetic efforts are focusing on designing molecules that mimic natural products, such as the preparation of FDA-approved fifth-generation β-lactams (i.e., ceftobiprole) as well as polymeric analogs of AMPs.[6−11] In polymer scenarios, most polymer structures are not degradable, which can limit their use in certain biological applications and lead to long-term resistance challenges.[12] This highlights the need for developing macromolecules that combine antimicrobial activity and the ability to degrade.

One class of macromolecules that has attracted less attention is depsides. They are natural compounds produced by condensation between hydroxybenzoic acids and have shown antimicrobial activity as a class.[13] For example, lecanoric acid, which is a depside of orsellinic acid (Scheme 1a), possesses antibacterial activity against Gram-negative and Gram-positive bacteria.[14,15] Depsides are found in lichen, plants, and fungi.[16,17] Interestingly, they possess a range of other biological activities such as antitumor,[18] anti-inflammatory,[19,20] antifungal,[21] and antiviral[15,22] properties. They have also been found active against drug-resistant bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA).[21,23] Depsides are linked by ester bonds, making them good candidates for preparing active and degradable molecules. However, the specificity that living organisms possess to link them at the 4-hydroxy position and the versatility of different carbon chain lengths at the 6-position pose synthetic challenges for researchers. Thus, preparing synthetic polymer analogs of depsides is an area of interest for drug discovery and biomedical applications.

Inspired by the promising potential of depsides, this study investigates the antimicrobial activity of hydroxybenzoate oligomers and polymers obtained using a combination of synthetic biology and chemical synthesis to enable access to a

Received: March 19, 2025
Revised: April 28, 2025
Accepted: April 30, 2025

Scheme 1. Polymeric Antimicrobial Agents against *S. aureus* Inspired by Depsides[a]



[a](a) Structure of a depside from orsellinic acid, (b) 4-(methyl/allyl/benzyl)oxy and 6-(H/alkyl)-2-hydroxybenzoates from synthetic and biological processes, and (c) their functional hydroxybenzoate-co-lactide polymers prepared by ROP.

range of functionalities (Scheme 1). Substituted hydroxybenzoic acids (such as resorcylic acids) are widely found in nature and are biosynthesized from polyketide pathways. Variations in the structure of these natural products arise through polyketide synthase substrate specificity and have been leveraged for engineered biosynthesis from model microbial hosts.[24] In this work, 2-hydroxybenzoic acid monomer precursors with 4-O (R_1) and 6-alkyl (R_2) functionality were heterologously produced and purified from an engineered fungal host.[25] The biosynthetically sourced natural products were studied for activity against Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Escherichia coli* (*E. coli*). These compounds were derivatized with methyl, allyl, and benzyl groups at the 4-O position and converted into the corresponding lactides prior to polymerization. The antimicrobial activity and biocompatibility of the resulting polymers demonstrate the importance of the peripheral functional group and molecular weight on antimicrobial efficacy. These data demonstrate the first successful design of functionalized poly(hydroxybenzoate-*co*-lactide) derivatives for antimicrobial activity.

RESULTS

Antimicrobial Screening of Small Molecules. While a few compounds such as salicylic acid (**1**), 2, 4-dihydroxybenzoic acid (resorcylic acid, **2**), and orsellinic acid (**6**) can be sourced commercially, those with longer 6-alkyl substitutions are not available (Figure 1). We previously described

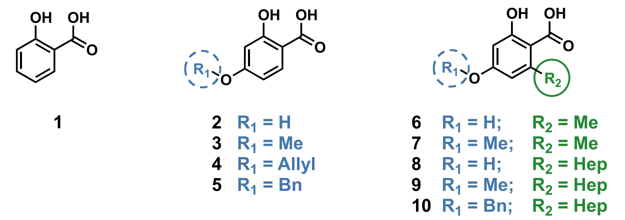


Figure 1. Compounds tested for antimicrobial evaluation with different R_1 and R_2 groups on the aromatic ring.

the discovery of biosynthetic pathways that produce the heptyl-substituted sphaerophorolcarboxylic acid (**8**).[25] Here, the microbial biosynthesis of **8** was scaled up from engineered *Aspergillus nidulans* for use as a building block (see ESI for details).

To study the effects of substitution on antimicrobial activity, we used resorcylic (**2**), orsellinic (**6**), and sphaerophorolcarboxylic (**8**) acids and introduced methyl, allyl, and benzyl groups at the 4-hydroxy position. This resulted in a diverse series of 2-hydroxybenzoic acids with varying substitutions at the 4-O (R_1) and 6 (R_2) positions (Figure 1, **compounds 1–10**, see ESI for detailed synthesis, Figures S1–S17). The small library of synthesized compounds was tested for activity against the Gram-positive organism *S. aureus* (strain Newman) and the Gram-negative organism *E. coli* (see Table 1).

Table 1. Antimicrobial Screening of 4-(Methyl/allyl/benzyl)oxy, 6-(H/alkyl)-2-hydroxybenzoates (Data Shown Are the Average of n = 3; When No Standard Deviation is Given, All Replicates Gave Identical MIC Values)

compound	*S. aureus* MIC (μg/mL)	*S. aureus* MIC (μM)	*E. coli* MIC (μg/mL)	*E. coli* MIC (μM)
1	62.5	453	375 ± 177	2715
2	1000	6488	188 ± 88	1217
3	1000	5947	>1000	>5947
4	500	2575	>1000	>5150
5	125	557	>1000	>4459
6	1000	5947	1000	5947
7	500	2745	1000	5489
8	62.5	248	250	991
9	15.6	59	250	939
10	3.9	11	>1000	>2920
ampicillin	1.0	3	31.3	61

The effect of the substituents with H-, methyl-, allyl-, or benzyl- groups at the 4-O position and methyl or heptyl groups at the 6- position on the aromatic ring was evaluated using a standard broth microdilution method to determine the minimum inhibitory concentration (MIC).[26] 4-O substituents (**2**–**5**) resulted in generally lower activity compared to **1**, although an increase in substituent size or hydrophobicity gave relatively higher antimicrobial activity. Specifically, small 4-O substituents generally decreased activity relative to salicylic acid, but a benzyl substituent retained antimicrobial activity

https://doi.org/10.1021/jacs.5c04624
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

(compound **5**, MIC = 125 μg/mL against *S. aureus*). On the other hand, when varying the alkyl side chain at the 6- position and keeping the 4- position constant (**8** vs **6** and **9** vs **7**), the 6-heptyl compounds (**8** and **9**) showed 16- to 32-fold lower MICs on *S. aureus* compared to the 6-methyl compounds (**6** and **7**), indicating that greater hydrophobicity at this position resulted in greater activity. The combination of a benzyl group at the 4-O position and a heptyl group at the 6- position (**10**) exhibited the highest antibacterial activity (MIC = 3.9 μg/mL), comparable to that of the positive control, ampicillin. Moreover, compound **10** showed antimicrobial activity against two other strains of *S. aureus*, including a methicillin-resistant *S. aureus* strain (MRSA; MIC = 15.6 μg/mL, Table S9). These compounds showed low or negligible activity against *E. coli*, suggesting that their effect may be related to the cell membrane.[27,28] With these results, monomers were then synthesized to enable the ring-opening polymerization of these adducts.

Monomer Preparation. One way to prepare oligomeric and polymeric analogs of depsides could be through polymerization from hydroxybenzoates. The preparation of the monomer was inspired by recent works dealing with the synthesis of alternate polymers consisting of salicylic acid and lactide. In the studies, the authors have prepared 3-methyl-5*H*-benzo[*e*][1,4]dioxepine-2,5(3*H*)-dione and 5*H*-benzo[*e*][1,4]-dioxepine-2,5(3*H*)-dione, which were further ring-opened using catalytic systems employing tin(II) 2-ethylhexanoate ($SnOct_2$),[29] 4-dimethylaminopyridine (DMAP),[30] or tetrabutylammonium halides[31] at a variety of temperatures (50 °C, 110−140 °C). Thus, the monomers were prepared similarly to previous works[29−34] with some modifications by reacting 4-(methyl/allyl/benzyl)oxy-6-alkyl-2-hydroxybenzoates with 2-bromo-propionyl-bromide (1.3 equiv) and triethylamine (TEA, 2.4 equiv, Figure 2a, compounds **11**−**15**, see ESI for synthesis details). Upon isolation, the monomers were obtained in 17−73% yields and characterized by spectroscopic techniques, including ^{1}H and ^{13}C NMR, microcrystal electron diffraction (MicroED), and X-ray crystallography (Figures S18−S31, Tables S1−S6). As a representative example, Figure 2b−g shows the characterization of the MicroED structure obtained for **13** with an excellent agreement between the ab initio structures determined by MicroED and single-crystal X-ray diffraction (SCXRD) (Figure 2g). While SCXRD and MicroED provided the same structural insights (Figures S22, S25, S29), MicroED could be performed on powders bearing nanoscale crystals.[35,36] This was much simpler for sample preparation compared to more traditional single-crystal X-ray diffraction, which required large single crystals.

Polymer Preparation. Although lactide monomers from salicylic acid are known to undergo ring-opening polymerization using $SnOct_2$, DMAP, tetrabutylammonium chloride,[29−34] and organometallic catalysts,[37] ROP using organocatalysts such as urea has not yet been reported. In this study, we explored the ROP of lactide monomers with 3-methylbutan-1-ol using the 3-O urea catalyst (1,1′,1″-(nitrilotris(ethane-2,1-diyl))tris(3-(3,5-bis(trifluoromethyl)-phenyl)urea)) and MTBD (1-methyl-2,3,4,6,7,8-hexahydro-1H-pyrimido[1,2-*a*]pyrimidine) as a cocatalyst in dichloromethane (DCM) under an inert atmosphere. An initial successful attempt using monomer **13** was conducted while targeting a ratio of 50:1:1:1 (monomer:3-O urea:MTBD:initiator), as confirmed by SEC and NMR spectroscopic studies (Figures 3 and S32−S34).

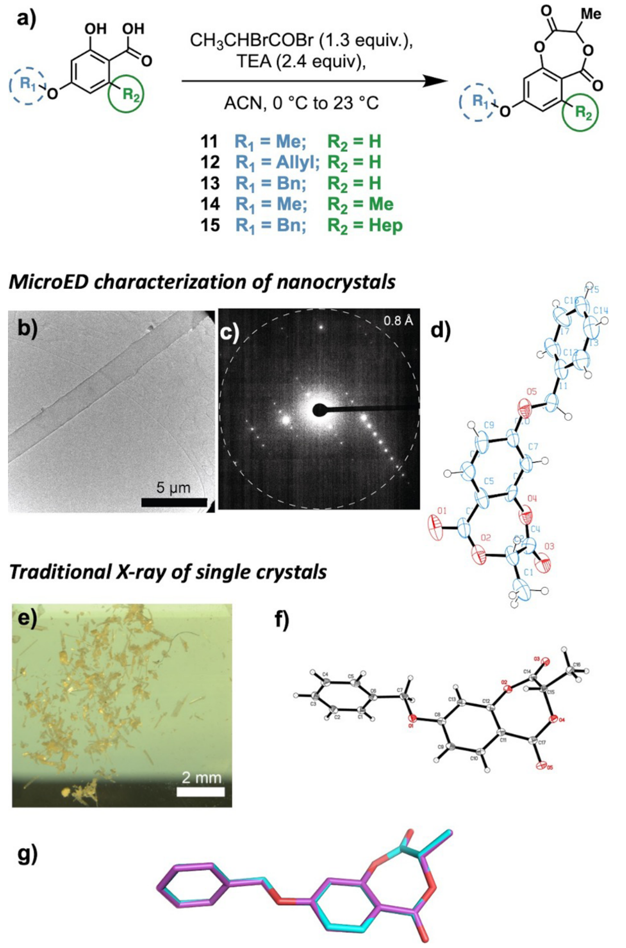


Figure 2. Preparation of lactide monomers (compounds **11**−**15**) from 4-(methyl/allyl/benzyl)oxy-6-(H/alkyl)-2-hydroxybenzoates and structure determination via MicroED from small crystallites: (a) synthesis of monomers; (b) nanocrystals of compound **13** that (c) yield high-resolution diffraction by MicroED, (d) such that their structures can be readily determined; and the structure of the same molecule determined from (e) larger crystals by (f) X-ray diffraction SCXRD; and (g) superimposed structures from MicroED and SCXRD all-atom reported with root-mean-square deviation (RMSD) of 0.028 Å.

The polymers analyzed by SEC in THF exhibited an M_n = 16.6 kg/mol (Figure 3b), which was slightly lower than the NMR calculation (20 kg/mol, monomer initiator concentration ratio, M/I = 66, Figure S32b), and the molar-mass dispersity was found to be $Đ$ = 1.14. On the other hand, using only MTBD or the 3-O urea catalyst, with or without DMAP, resulted in higher dispersities, and no polymerization was observed in the absence of a catalyst (Table S7). The results suggested that it is feasible to prepare polymers with a targeted M/I by controlling the stoichiometric ratio of the monomer, catalyst, cocatalyst, and initiator. To validate this, the monomer initiator concentration ratio (M/I) for **13** was varied from 30 to 90 (Figure 3b), and a linear relationship was observed from this assay while preserving low dispersities ($Đ$ < 1.3, Figure 3c, Table S8). Thus, it was possible to precisely control the M/I by varying the monomer/initiator ratio using the organocatalytic system.

Molecular weight is an important parameter in the design of antimicrobial polymers, particularly depsides are found as oligomeric compounds.[17] In the literature, most active

https://doi.org/10.1021/jacs.5c04624
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

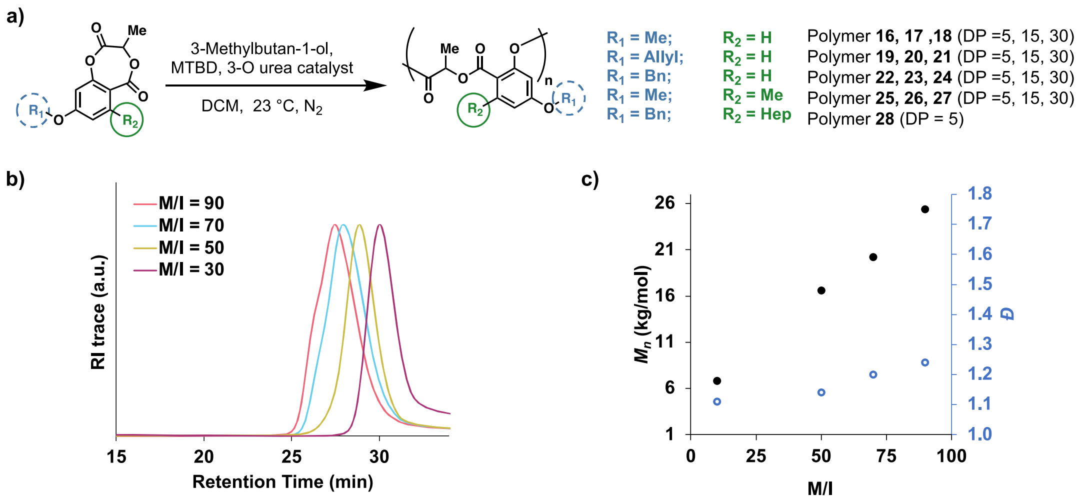


Figure 3. Preparation and characterization of polymers derived from 4-(methyl/allyl/benzyl)-oxy-6-alkyl-2-oxybenzoate lactide monomers by ring-opening polymerization. (a) Synthetic route to prepare polymers with different M/I. Variation of the [monomer]/[initiator] (M/I) during ROP of monomer **13**: (b) refractive index (RI) trace SEC in THF and (c) plot of M_n (black) and dispersity ($Đ$, blue) versus M/I.

polymers have an M/I ≤ 30, although high molecular weight polymers can also exhibit antimicrobial activity.[38−40] Thus, after the successful polymerization of **13**, a series of polymers were prepared with different substituents and degrees of polymerization (M/I = 5, 15, and 30) (Figure 3a). The polymerizations went to completion, and the polymers were isolated in 53−91% yields and analyzed by NMR spectroscopy and SEC (Table 2 and Figures S35−S61). The majority of the obtained polymers aligned well with the targeted polymerization degree. At low polymerization degrees, we observed multiple peaks in both the ^{1}H NMR and ^{13}C NMR spectra attributed to the oligomeric forms and uncontrolled stereoregularity. Furthermore, polymers synthesized with an M/I = 30 were examined by ^{13}C NMR spectroscopy, and no evidence of transesterification was observed,[29] although this cannot be completely ruled out. Moreover, the M_n obtained from SEC analysis was in full agreement with the calculated M/I from ^{1}H NMR analysis for M/I = 30 and possessed M_n = 5.6−6.8 kg/mol and $Đ$ = 1.11−1.23. For M/I < 30, it was not possible to conduct SEC analysis due to instrument limitations. Therefore, the degree of polymerization was determined by ^{1}H NMR spectroscopy.

Table 2. Characterization of Functional Hydroxybenzoate-co-Lactide Polymers with Different M/I

polymer	M/I_{theo}	M_n (kg/mol)[a]	$Đ$	M/I_{NMR}	yield (%)
16	5			5	84
17	15			13	91
18	30	6.1	1.23	30	84
19	5			5	59
20	15			11	81
21	30	5.5	1.10	22	58
22	5			6	61
23	15			10	49
24	30	6.8	1.11	30	89
25	5			7	53
26	15			12	44
27	30	5.6	1.21	28	78
28	5			3	63

[a]Determined from SEC in THF.

Antimicrobial Assays of Polymers. Next, we evaluated the antimicrobial potency of the polymer library against *S. aureus* by determining the minimum inhibitory concentration (MIC) using the same broth microdilution protocol as that for the small molecules. Interestingly, some polymers displayed enhanced antibacterial activity (Table 3) compared with their small molecule forms (Table 1). Comparison of the substituent variation on the 4-O position with M/I_{NMR} < 10 showed significant differences among the methyl, allyl, or benzyl side chains when assessing the MIC values of polymers **16** (MIC = 333 ± 144 μg/mL), **19** (MIC = 1000 μg/mL), and **22** (MIC = 83 ± 36 μg/mL), with the benzyl substituent resulting in the greatest activity for this position, similar to the small molecules. Interestingly, when a methyl group was introduced at the 6-position in **25**, the polymer displayed a higher antimicrobial effect (MIC = 31.3 μg/mL), compared to its small molecule counterpart (Table 1, compound **7**). Moreover, introducing R_1 = Bn, and R_2 = heptyl, which had given the greatest activity among the small molecules (compound **10**), led to polymer **28** with antimicrobial potency similar to that of **25** (MIC = 31.3 μg/mL). Thus, small molecule activity was not always a good predictor of polymer activity.

The second comparison assessed the effect of M/I on antimicrobial activity (Table 3). It was observed that increasing the M/I above 5 increased the MIC values for all polymers. A representative example is the loss of activity on polymer **25**, whose MIC significantly increased from 31 μg/mL at M/I = 7 to >1000 μg/mL at M/I = 28 (polymer **27**). Together, these results demonstrate the antibacterial activity against Gram-positive microorganism *S. aureus*, highlighting the importance of the substituents at 4-O and 6- positions and the size of the hydroxybenzoate polymers.

One major concern with bacteria is their ability to form biofilms, which are complex matrices that confer an extra layer of protection and prevent the penetration of antibiotics.[41,42] Thus, disruption and inhibition of biofilms are important targets for combating bacteria. We hypothesized that the active polymers **22**, **25**, and **28** prepared in this work could also inhibit and disrupt biofilms. To assess this hypothesis, the inhibition of biofilm was assessed in *S. aureus* cultures under stationary conditions with different concentrations of the polymers for 72 h in a 96-well plate. For biofilm disruption

https://doi.org/10.1021/jacs.5c04624
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

Table 3. Antimicrobial Activity and MTT Assay on *S. aureus* of Polymers M/I = 5−30 (n = 3)

polymer	R_1	R_2	M/I_{NMR}	*S. aureus* MIC (μg/mL)	*S. aureus* MIC (μM)[a]	cell viability fibroblasts ≥80% (CC_{80}, μg/mL)[b]	cell viability fibroblasts ≥80% (CC_{80}, μM)[c]	selectivity index (SI)[d]
16	Me	H	5	333 ± 144	278 ± 98	1000	834	3
17	Me	H	13	>1000	>336	1000	336	<1
18	Me	H	30	>1000	>148	1000	148	<1
19	allyl	H	5	1000	752	500	376	0.5
20	allyl	H	11	>1000	>355	1000	355	<1
21	allyl	H	22	>1000	>180	1000	180	<1
22	Bn	H	6	83 ± 36	44 ± 16	1000	533	12
23	Bn	H	10	>1000	>326	1000	326	<1
24	Bn	H	30	>1000	>111	1000	111	<1
25	Me	Me	7	31.3	18	1000	574	32
26	Me	Me	12	>1000	>342	1000	342	<1
27	Me	Me	28	>1000	>149	1000	149	<1
28	Bn	Hep	3	31.3	25	50	39	2
ampicillin				1.0	3	1000		

[a]Calculated from the M_w from ^{1}H NMR. [b]The cell viability was determined from the MTT assay. [c]The cell viability at ≥80% in μM was determined using the M/I_{NMR} of each polymer. [d]Calculated from MIC over CC_{80}

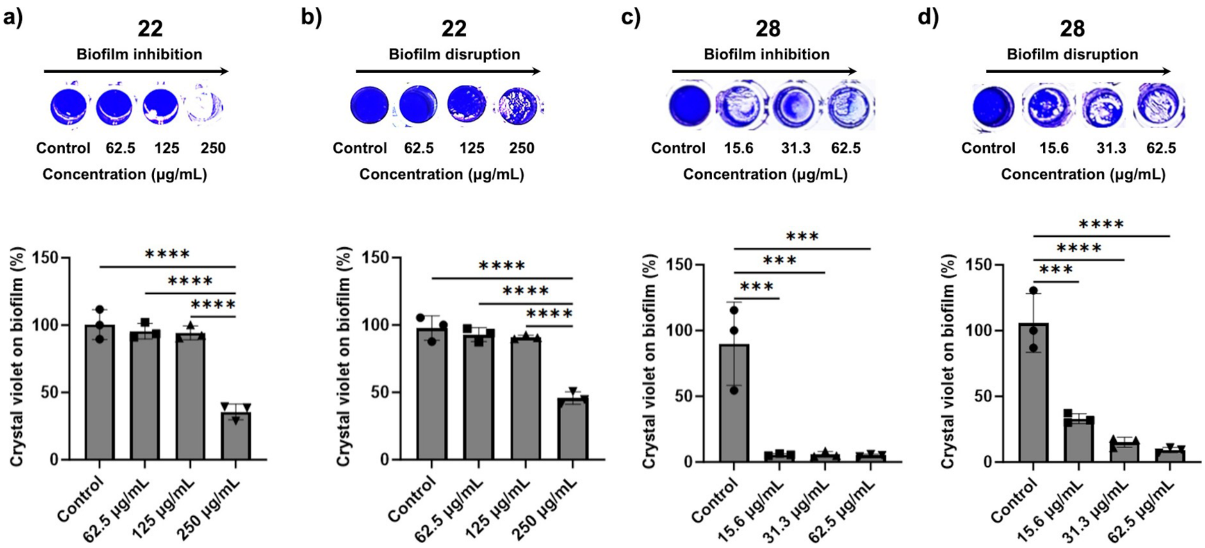


Figure 4. Biofilm inhibition and disruption of functional hydroxybenzoate-co-lactide polymers **22** (a, b) and **28** (c, d) on *S. aureus*. Antimicrobial properties were determined by evaluating the biofilm inhibition and disruption by staining with 1% crystal violet. The figure shows the 96-well plate picture accompanied by the UV−vis quantitation of the biofilm assays. Statistical significance was determined via a one-way ANOVA with multiple comparisons (p < 0.05 (*), p < 0.01 (**), p < 0.001 (***), p < 0.0001 (****)) (n = 3).

studies, *S. aureus* was cultured under stationary conditions for 34 h in a 96-well plate to form biofilms. After 34 h (once the biofilm was formed), each well was treated with different concentrations of the synthesized polymers and incubated for an additional 38 h. After 72 h, the effect on biofilm inhibition and disruption was observed by staining the well with crystal violet dye, which binds the extracellular polymeric substance (EPS) of the biofilm (see the ESI). Polymers **22**, **25**, and **28** were tested along with their small molecule precursor **5**, **7**, and **10**, respectively (Figures 4 and S62). Interestingly, inhibition of biofilm and disruption were observed for polymer **22** at 250 μg/mL (Figure 4a,b) and for **28** at 15.6 μg/mL. However, polymer **25** did not display biofilm inhibition or disruption at 2- or 3-fold higher concentrations (Figure S62) even though it exhibited antimicrobial activity in liquid culture.

Antibiofilm activity was also compared between the corresponding small molecules and polymers. Compound **5** (precursor of **22**) did not cause biofilm disruption or inhibition, and compounds **7** (precursor of **25**) and **10** (precursor of **28**) displayed both inhibition and disruption of the *S. aureus* biofilm (Figure S62). Of these, compound **7** showed a weaker effect, with inhibition at 250 μg/mL and disruption at 1000 μg/mL; while compound **10** showed relatively potent inhibition at 2 μg/mL and disruption of biofilm at 7.8 μg/mL.

We also evaluated the particle size formation of the polymers by dynamic light scattering (DLS) as they formed turbid solutions during the activity tests described above at 1000 μg/mL (Table S10). Despite the formation of self-assemblies within the range of 117−686 nm, no correlation was found between antimicrobial activity and particle size. These results demonstrated that the inhibition and disruption of biofilm depends on the molecular weight and chemical structure.

Biocompatibility Studies. To evaluate the suitability of the polymers, cell viability on NIH 3T3 fibroblasts was determined using an MTT assay. The results demonstrated

https://doi.org/10.1021/jacs.5c04624
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

low toxicity as the cell viability was ≥80% (CC_{80}) for all tested polymers at ≥500 μg/mL, except for polymer **28**, which was cytotoxic at CC_{80} = 50 μg/mL (Table 3, and Figure S63). This could be due to the increase in hydrophobicity of this polymer. Representative examples of cell viability for polymers **22** and **24** are presented in Figure 5; for these polymers, the

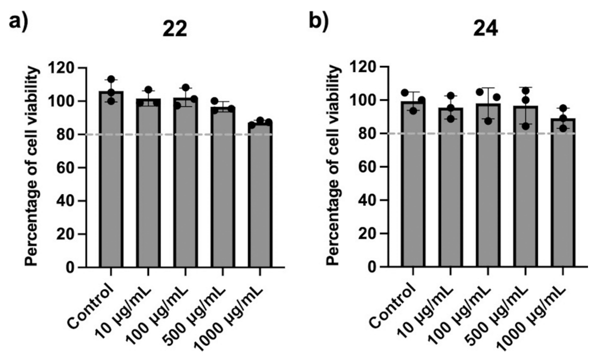


Figure 5. Representative examples of the in vitro biocompatibility evaluated in the NIH 3T3 fibroblast cell line by MTT assay for polymers **22** and **24** (a, b) having M/I = 6 and 30, respectively. Statistical significance was determined via a one-way ANOVA with multiple comparisons ($p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****)) (n = 3).

cytotoxicity was low as the cell viability was ≥80% even at 1000 μg/mL at different M/I. When comparing the cell viability of all the polymers to their precursors (compounds **1**−**10**, see Figure S64), the polymers displayed higher cell viability (>80% cell viability, Table 3 and Figure S64). However, the introduction of the heptyl side chain could increase interactions with the lipid bilayer of eukaryotic cells.

In order to estimate how selective the polymers were between bacterial and mammalian cells, the selectivity index (SI) was calculated as the ratio of the concentration that results in cell viability ≥80% (CC_{80}) and the MIC value (SI = CC_{80}/MIC) for each polymer tested (Table 3). The results showed the highest selectivity for the polymer derived from methylated orsellinic acid (R_1 and R_2 = Me) groups at M/I = 7 (polymer **25**, SI = 32). The active polymer **22** presented a reduced, yet acceptable SI of 12, demonstrating that increasing the hydrophobicity on the side chains may decrease selectivity toward eukaryotic cells, as seen for polymer **28** (SI = 2). Together, these results demonstrated that most of the polymers prepared in this work possess low toxicity, and two show SI ≥ 12.

Degradability. To assess the degradability potential of these polymers, degradation of polymers that were able to form films (M/I = 30) through solution casting **18**, **21**, **24**, and **27** was studied and compared to known degradable polymers, including poly(D,L-lactic acid-glycolic acid) random distribution 50−50 (P(DLLA-*r*-GA)), alternating 50−50 (P(LLA-*alt*-GA)), and poly(D,L-lactic acid) (P(DLLA)). The experiments were conducted at 50 °C in 0.1 M sodium hydroxide (NaOH) solution, and the degradation profiles were compared (Figure 6). The polymers with R_1 = CH_3 and allyl (**18** and **21**) exhibited similar degradation profiles and are comparable to P(LLA-*alt*-GA), whereas P(DLLA-*r*-GA) and P(DLLA) degraded at a faster rate. The polymer with Bn at the 4-O position (**24**) showed the slowest degradation rate. Therefore, the results show that the polymers degrade as fast or slightly slower than commonly used biodegradable polymers, suggesting that the rate does not depend on the substituents at the molecular weight range studied (<10 kg/mol). Degradation under more physiological conditions (37 °C in PBS pH = 7.4) was also determined. As expected, the degradation rate was slower, with more than 65% remaining after ∼1 month (Figure S65). It should be noted that various enzymes can degrade polyesters; therefore, the rates in vivo may be much faster than in PBS.

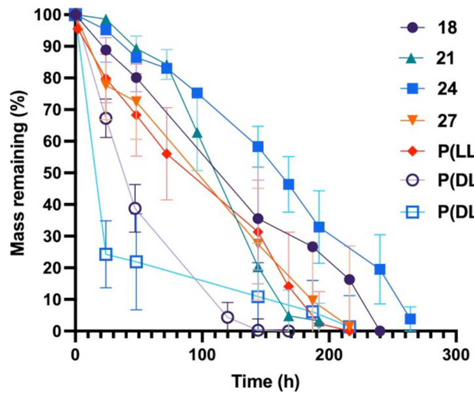


Figure 6. Degradation of polymers studied at 50 °C in 0.1 M NaOH (n = 3).

■ DISCUSSION

Inspired by active natural products, the antimicrobial potential of functional hydroxybenzoate-*co*-lactide polymers was explored. The synthesis of the precursors using a systems biology approach, easy evaluation of the monomer structures by MicroED, and a controlled organocatalytic ROP approach facilitated the rapid production of a diverse library of oligomers and polymers with targeted molecular weights.

The structure−activity relationship study of the precursors demonstrated that modifications at the 4-O and 6-positions of the aromatic ring significantly enhanced activity against *S. aureus*, with the 6-heptyl series exhibiting the lowest MICs (Figure 7 for a summary).

With regard to antimicrobial activity, the polymers were more active in general at smaller sizes; degrees of polymerization at or below 7 were most active within their respective series. The effect of molecular weight was crucial, as polymers with M/I > 10 were inactive. Moreover, the effect of the substituents was important for biofilm inhibition and disruption, as polymers with R_1 = Bn and R_2 = H (**22**) or R_1 = Bn and R_2 = Hep (**28**) showed both inhibition and disruption of the biofilm, whereas the polymer with R_1 = Me and R_2 = Me did not show antibiofilm activity (**25**). The polymers were also tested for cell viability, leading to two potentially biocompatible candidates with SI ≥ 12 (polymers **22** and **25**). A summary of the polymer activity and selectivity is provided in Figure 7. Notably, the polymers demonstrated degradability generally comparable to poly(lactic acid). Combining degradability with the antimicrobial properties, these polymeric platforms are interesting for future preparation of long-lasting antimicrobials, especially because both the oligomeric forms and the precursors are active. The results demonstrate the benefit of (a) preparing polymers of hydroxybenzoates and (b) modifying the ring. Additional studies are needed to elucidate the exact mechanism, but it is clear that a balance of hydrophobic side chains is required.[43] In cases in which the polymers are more active than the

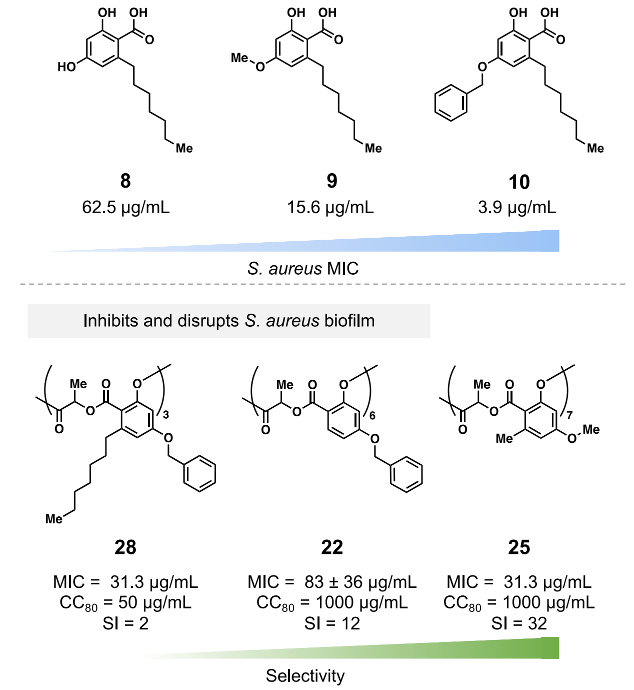


Figure 7. Key compounds with antimicrobial activity against *S. aureus*. MIC is the minimal inhibitory concentration against *S. aureus*, CC_{80} is the concentration at which fibroblast cell viability ≥80%, and SI is the selectivity index. Polymers **22** and **28** inhibit and disrupt *S. aureus* biofilms.

monomers, the difference may be due to effects such as cooperativity and avidity, or the polymer and monomer may act through different mechanisms; understanding these mechanisms will require further research.

Together, these results confirm the potential of modifying antibacterial properties by designing polymers with easy-to-functionalize hydroxybenzoate groups. Potential applications include the preparation of materials with long-lasting antibacterial effects through the degradation and release of small active ingredients, such as for coatings of medical devices or fibers for antimicrobial wound dressings. To realize these biomedical applications, a fine evaluation of oligomer size along with future studies involving extensive evaluation of in vivo biocompatibility and efficacy needs to be addressed.

CONCLUSIONS

In this work, we prepared depside-inspired antimicrobial oligomers and polymers derived from hydroxybenzoate-co-lactide bearing different substituents, by combining synthetic biology and polymer chemistry. These macromolecules, including their precursors, displayed antimicrobial activity toward *S. aureus*. Our findings indicate that these well-defined polymers are promising candidates for treating Gram-positive *S. aureus* infections and their biofilms. The low cytotoxicity effects suggest that high selectivity indexes can be reached using the polymeric platforms. Thus, this work demonstrates the potential of using degradable synthetic materials with antimicrobial properties that can be implemented for biomedical purposes.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.5c04624.

Materials and methods, NMR, SEC, crystallography, and biological experiments (PDF)

Accession Codes

Deposition Numbers 2401549−2401550 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via the joint Cambridge Crystallographic Data Centre (CCDC) and Fachinformationszentrum Karlsruhe Access Structures service.

AUTHOR INFORMATION

Corresponding Authors

Irene A. Chen − *Department of Chemistry and Biochemistry, BioPACIFIC Materials Innovation Platform California NanoSystems Institute, and Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, California 90095, United States;* orcid.org/0000-0001-6040-7927; Email: ireneachen@ucla.edu

Javier Read de Alaniz − *Department of Chemistry and Biochemistry and BioPACIFIC Materials Innovation Platform California NanoSystems Institute, University of California, Santa Barbara, California 93106, United States;* orcid.org/0000-0003-2770-9477; Email: jalaniz@ucsb.edu

Heather D. Maynard − *Department of Chemistry and Biochemistry and BioPACIFIC Materials Innovation Platform California NanoSystems Institute, University of California, Los Angeles, California 90095, United States;* orcid.org/0000-0003-3692-6289; Email: maynard@chem.ucla.edu

Authors

Pedro Salas-Ambrosio − *Department of Chemistry and Biochemistry and BioPACIFIC Materials Innovation Platform California NanoSystems Institute, University of California, Los Angeles, California 90095, United States;* orcid.org/0000-0002-0922-4620

Shelby Vexler − *Department of Chemistry and Biochemistry and BioPACIFIC Materials Innovation Platform California NanoSystems Institute, University of California, Los Angeles, California 90095, United States;* orcid.org/0000-0001-7844-5390

Rajalakshmi P Sivasankaran − *BioPACIFIC Materials Innovation Platform California NanoSystems Institute, University of California, Los Angeles, California 90095, United States;* orcid.org/0000-0002-7781-055X

Niko Vlahakis − *Department of Chemistry and Biochemistry and BioPACIFIC Materials Innovation Platform California NanoSystems Institute, University of California, Los Angeles, California 90095, United States*

Ryan S. Lai − *Department of Chemistry and Biochemistry and BioPACIFIC Materials Innovation Platform California NanoSystems Institute, University of California, Los Angeles, California 90095, United States*

Colin Johnson − *Department of Chemistry and Biochemistry and BioPACIFIC Materials Innovation Platform California NanoSystems Institute, University of California, Los Angeles, California 90095, United States;* orcid.org/0000-0003-1092-1172

Sophia I. Baas-Maynard − *Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, United States*
Daniel S. Min − *Department of Chemistry and Biochemistry and BioPACIFIC Materials Innovation Platform California NanoSystems Institute, University of California, Los Angeles, California 90095, United States*
Haillie Lower − *Department of Chemistry and Biochemistry and BioPACIFIC Materials Innovation Platform California NanoSystems Institute, University of California, Los Angeles, California 90095, United States*
Abigail G. Doyle − *Department of Chemistry and Biochemistry and BioPACIFIC Materials Innovation Platform California NanoSystems Institute, University of California, Los Angeles, California 90095, United States;* orcid.org/0000-0002-6641-0833
Yi Tang − *Department of Chemistry and Biochemistry, BioPACIFIC Materials Innovation Platform California NanoSystems Institute, and Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, California 90095, United States*
Jose A. Rodriguez − *Department of Chemistry and Biochemistry and BioPACIFIC Materials Innovation Platform California NanoSystems Institute, University of California, Los Angeles, California 90095, United States;* orcid.org/0000-0002-0248-4964

Complete contact information is available at:
https://pubs.acs.org/10.1021/jacs.5c04624

Author Contributions

⊥S.V. and R.P.S. contributed equally to this work.

Funding

This work was funded by the National Science Foundation BioPACIFIC Materials Innovation Platform (DMR-1933487). The authors also thank the Dr. Myung K_i Hong Chair in Polymer Science (HDM) and National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers T32GM145388, R35GM148249, 2R35GM12886706, and DP2GM123457 (IAC). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. R.S.L. and H.L. thank the Department of Chemistry and Biochemistry and the Dean of Physical Sciences at UCLA for funding.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to thank the scientists for assistance in different instrumentation: Saeed Khan (X-ray monocrystals), Ignacio Martini (SEC), Roberto Chavez, Robert Peterson, Ta-Chung Ong (NMR); Gregory Khitrov, Yu Chen (MS).

■ REFERENCES

(1) *2019 Antibiotic Resistance Threats Report | CDC*. https://www.cdc.gov/drugresistance/biggest-threats.html (accessed Dec 11, 2024).
(2) Pham, P.; Oliver, S.; Boyer, C. Design of Antimicrobial Polymers. *Macromol. Chem. Phys.* **2023**, *224* (3), No. 2200226.
(3) Fleming, A. On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to Their Use in the Isolation of B. Influenzæ. *Br. J. Exp. Biol.* **1929**, *10* (3), 226−236.
(4) Schmidt, N. W.; Mishra, A.; Lai, G. H.; Davis, M.; Sanders, L. K.; Tran, D.; Garcia, A.; Tai, K. P.; McCray, P. B.; Ouellette, A. J.; Selsted, M. E.; Wong, G. C. L. Criterion for Amino Acid Composition of Defensins and Antimicrobial Peptides Based on Geometry of Membrane Destabilization. *J. Am. Chem. Soc.* **2011**, *133* (17), 6720−6727.
(5) Lee, E. Y.; Fulan, B. M.; Wong, G. C. L.; Ferguson, A. L. Mapping Membrane Activity in Undiscovered Peptide Sequence Space Using Machine Learning. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (48), 13588−13593.
(6) Nederberg, F.; Zhang, Y.; Tan, J. P. K.; Xu, K.; Wang, H.; Yang, C.; Gao, S.; Guo, X. D.; Fukushima, K.; Li, L.; Hedrick, J. L.; Yang, Y. Y. Biodegradable Nanostructures with Selective Lysis of Microbial Membranes. *Nat. Chem.* **2011**, *3* (5), 409−414.
(7) Ilker, M. F.; Nüsslein, K.; Tew, G. N.; Coughlin, E. B.; Nu, K.; Tew, G. N.; Coughlin, E. B. Tuning the Hemolytic and Antibacterial Activities of Amphiphilic Polynorbornene Derivatives. *J. Am. Chem. Soc.* **2004**, *126* (48), 15870−15875.
(8) Xiong, M.; Lee, M. W.; Mansbach, R. A.; Song, Z.; Bao, Y.; Peek, R. M.; Yao, C.; Chen, L. F.; Ferguson, A. L.; Wong, G. C. L.; Cheng, J. Helical Antimicrobial Polypeptides with Radial Amphiphilicity. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112* (43), 13155−13160.
(9) Kuroda, K.; DeGrado, W. F. Amphiphilic Polymethacrylate Derivatives as Antimicrobial Agents. *J. Am. Chem. Soc.* **2005**, *127* (12), 4128−4129.
(10) Salas-Ambrosio, P.; Tronnet, A.; Since, M.; Bourgeade-Delmas, S.; Stigliani, J.-L.; Vax, A.; Lecommandoux, S.; Dupuy, B.; Verhaeghe, P.; Bonduelle, C. Cyclic Poly(α-Peptoid)s by Lithium Bis-(Trimethylsilyl)Amide (LiHMDS)-Mediated Ring-Expansion Polymerization: Simple Access to Bioactive Backbones. *J. Am. Chem. Soc.* **2021**, *143* (10), 3697−3702.
(11) Mowery, B. P.; Lindner, A. H.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. Structure-Activity Relationships among Random Nylon-3 Copolymers That Mimic Antibacterial Host-Defense Peptides. *J. Am. Chem. Soc.* **2009**, *131* (28), 9735−9745.
(12) Tuvo, B.; Scarpaci, M.; Bracaloni, S.; Esposito, E.; Costa, A. L.; Ioppolo, M.; Casini, B. Microplastics and Antibiotic Resistance: The Magnitude of the Problem and the Emerging Role of Hospital Wastewater. *Int. J. Environ. Res. Public Health* **2023**, *20* (10), 5868.
(13) Fischer, E. Synthesis of Depsides, Lichen-Substances and Tannins. *J. Am. Chem. Soc.* **1914**, *36* (6), 1170−1201.
(14) Rajendran, K.; Poornima, S.; Ponnusamy, P. Antimicrobial and Antiproliferative Activities of Depside Compound Isolated from the Mycobiont Culture of Parmotrema Austrosinense (Zahlbr.) Hale. *J. Pure Appl. Microbiol* **2020**, *14* (4), 2525−2541.
(15) Gomes, A. T.; Artur; Júnior, S.; Seidel, C.; De Fátima, E.; Smania, A.; Neli; Honda, K.; Fernanda; Roese, M.; Muzzi, R. M. Antibacterial Activity of Orsellinates. *Braz. J. Microbiol.* **2003**, *34*, 194−196.
(16) Ureña-Vacas, I.; González-Burgos, E.; Divakar, P. K.; Gómez-Serranillos, M. P. Lichen Depsides and Tridepsides: Progress in Pharmacological Approaches. *Journal of Fungi* **2023**, *9* (1), 116.
(17) Ibrahim, S. R. M.; Sirwi, A.; Eid, B. G.; Mohamed, S. G. A.; Mohamed, G. A. Fungal Depsides—Naturally Inspiring Molecules: Biosynthesis, Structural Characterization, and Biological Activities. *Metabolites* **2021**, *11* (10), 683.
(18) Zhou, R.; Yang, Y.; Park, S. Y.; Nguyen, T. T.; Seo, Y. W.; Lee, K. H.; Lee, J. H.; Kim, K. K.; Hur, J. S.; Kim, H. The Lichen Secondary Metabolite Atranorin Suppresses Lung Cancer Cell Motility and Tumorigenesis. *Sci. Rep.* **2017**, *7* (1), 8136.
(19) Kumar KC, S.; Müller, K. Lichen Metabolites. 1. Inhibitory Action against Leukotriene B4 Biosynthesis by a Non-Redox Mechanism. *J. Nat. Prod* **1999**, *62* (6), 817−820.
(20) Müller, K. Pharmaceutically Relevant Metabolites from Lichens. *Appl. Microbiol. Biotechnol.* **2001**, *56* (1−2), 9−16.
(21) Saetang, P.; Rukachaisirikul, V.; Phongpaichit, S.; Preedanon, S.; Sakayaroj, J.; Hadsadee, S.; Jungsuttiwong, S. Antibacterial and Antifungal Polyketides from the Fungus *Aspergillus Unguis* PSU-MF16. *J. Nat. Prod* **2021**, *84* (5), 1498−1506.
(22) Yılmaz, M.; Türk, A. Ö.; Tay, T.; Kıvanç, M. The Antimicrobial Activity of Extracts of the Lichen Cladonia Foliacea and Its (−)-Usnic

Acid, Atranorin, and Fumarprotocetraric Acid Constituents. *Zeitschrift für Naturforschung C* **2004**, *59* (3–4), 249–254.
(23) Phainuphong, P.; Rukachaisirikul, V.; Phongpaichit, S.; Sakayaroj, J.; Kanjanasirirat, P.; Borwornpinyo, S.; Akrimajirachoote, N.; Yimnual, C.; Muanprasat, C. Depsides and Depsidones from the Soil-Derived Fungus Aspergillus Unguis PSU-RSPG204. *Tetrahedron* **2018**, *74* (39), 5691–5699.
(24) Zhou, H.; Zhan, J.; Watanabe, K.; Xie, X.; Tang, Y. A Polyketide Macrolactone Synthase from the Filamentous Fungus *Gibberella Zeae*. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (17), 6249–6254.
(25) Okorafor, I. C.; Chen, M.; Tang, Y. High-Titer Production of Olivetolic Acid and Analogs in Engineered Fungal Host Using a Nonplant Biosynthetic Pathway. *ACS Synth. Biol.* **2021**, *10* (9), 2159–2166.
(26) Kowalska-Krochmal, B.; Dudek-Wicher, R. The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance. *Pathogens* **2021**, *10* (2), 165.
(27) Benito, M. J.; Tchounwou, P. B.; Song, X.; Li, R.; Zhang, Q.; He, S.; Wang, Y. Antibacterial Effect and Possible Mechanism of Salicylic Acid Microcapsules against Escherichia Coli and Staphylococcus Aureus. *J. Environ. Res. Public Health* **2022**, *19*, No. 12761.
(28) Bouarab, L.; Degraeve, P.; Bouajila, J.; Cottaz, A.; Jbilou, F.; Joly, C.; Oulahal, N. *Staphylococcus Aureus* Membrane-Damaging Activities of Four Phenolics. *FEMS Microbiol. Lett.* **2021**, *368* (13), No. fnab081.
(29) Kim, H. J.; Hillmyer, M. A.; Ellison, C. J. Enhanced Polyester Degradation through Transesterification with Salicylates. *J. Am. Chem. Soc.* **2021**, *143* (38), 15784–15790.
(30) Kim, H. J.; Reddi, Y.; Cramer, C. J.; Hillmyer, M. A.; Ellison, C. J. Readily Degradable Aromatic Polyesters from Salicylic Acid. *ACS Macro Lett.* **2020**, *9* (1), 96–102.
(31) Ren, F.; Xian, J.; Jia, Z.; Chen, Z.; Fu, H.; Wang, R.; Chu, W.; Pan, X.; Wu, J. Tetrabutylammonium Halides as Selectively Bifunctional Catalysts Enabling the Syntheses of Recyclable High Molecular Weight Salicylic Acid-Based Copolyesters. *Angew. Chem., Int. Ed.* **2023**, *62* (44), No. e202306759.
(32) Pedna, A.; Rosi, L.; Frediani, M.; Frediani, P. High Glass Transition Temperature Polyester Coatings for the Protection of Stones. *J. Appl. Polym. Sci.* **2015**, *132* (30), No. 42323.
(33) Baker, G.; Jing, F.; Smith, M. Degradable 1,4-Benzodioxepin-3-Hexyl-2,5-Dione Monomer Derived Polymer with a High Glass Transition Temperature. 2008/0146774 A1, June 19, 2008.
(34) Kim, H. J.; Peng, X.; Shin, Y.; Hillmyer, M. A.; Ellison, C. J. Blend Miscibility of Poly(Ethylene Terephthalate) and Aromatic Polyesters from Salicylic Acid. *J. Phys. Chem. B* **2021**, *125* (1), 450–460.
(35) Jones, C. G.; Martynowycz, M. W.; Hattne, J.; Fulton, T. J.; Stoltz, B. M.; Rodriguez, J. A.; Nelson, H. M.; Gonen, T. The CryoEM Method MicroED as a Powerful Tool for Small Molecule Structure Determination. *ACS Cent Sci.* **2018**, *4* (11), 1587–1592.
(36) Gruene, T.; Wennmacher, J. T. C.; Zaubitzer, C.; Holstein, J. J.; Heidler, J.; Fecteau-Lefebvre, A.; De Carlo, S.; Müller, E.; Goldie, K. N.; Regeni, I.; Li, T.; Santiso-Quinones, G.; Steinfeld, G.; Handschin, S.; van Genderen, E.; van Bokhoven, J. A.; Clever, G. H.; Pantelic, R. Rapid Structure Determination of Microcrystalline Molecular Compounds Using Electron Diffraction. *Angew. Chem., Int. Ed.* **2018**, *57* (50), 16313–16317.
(37) Fastnacht, K. V.; Spink, S. S.; Dharmaratne, N. U.; Pothupitiya, J. U.; Datta, P. P.; Kiesewetter, E. T.; Kiesewetter, M. K. Bis- and Tris-Urea H-Bond Donors for Ring-Opening Polymerization: Unprecedented Activity and Control from an Organocatalyst. *ACS Macro Lett.* **2016**, *5* (8), 982–986.
(38) Salas-Ambrosio, P.; Vexler, S.; Rajalakshmi, P. S.; Chen, I. A.; Maynard, H. D. Caffeine and Cationic Copolymers with Antimicrobial Properties. *ACS Bio Med. Chem. Au* **2023**, *3* (2), 189–200.
(39) Kanazawa, A.; Ikeda, T.; Endo, T. Antibacterial Activity of Polymeric Sulfonium Salts. *J. Polym. Sci. A Polym. Chem.* **1993**, *31* (11), 2873–2876.
(40) Weight, M.; Salts, P. P.; Kanazawa, A.; Ikeda, T.; Endo, T. Polymeric Phosphonium Salts as a Novel Class of Cationic Biocides. II. Effects of Counter Anion and Molecular Weight on Antibacterial Activity of Polymeric Phosphonium Salts. *J. Polym. Sci. A Polym. Chem.* **1993**, *31* (6), 1441–1447.
(41) Flemming, H. C.; van Hullebusch, E. D.; Neu, T. R.; Nielsen, P. H.; Seviour, T.; Stoodley, P.; Wingender, J.; Wuertz, S. The Biofilm Matrix: Multitasking in a Shared Space. *Nature Reviews Microbiology 2022 21:2* **2023**, *21* (2), 70–86.
(42) Kolpen, M.; Jensen, P. Ø.; Faurholt-Jepsen, D.; Bjarnsholt, T. Prevalence of Biofilms in Acute Infections Challenges a Longstanding Paradigm. *Biofilm* **2022**, *4*, No. 100080.
(43) Jung, K.; Corrigan, N.; Wong, E. H. H.; Boyer, C. Bioactive Synthetic Polymers. *Adv. Mater.* **2022**, *34* (2), No. 2105063.

https://doi.org/10.1021/jacs.5c04624
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX