

# UNIVERSITY OF BIRMINGHAM

University of Birmingham  
Research at Birmingham

## Antimicrobial hyperbranched polymer-usnic acid complexes through a combined ROP-RAFT strategy

Rauschenbach, Moritz; Lawrenson, Stefan B; Taresco, Vincenzo; Pearce, Amanda K; O'Reilly, Rachel K

DOI:

[10.1002/marc.202000190](https://doi.org/10.1002/marc.202000190)

License:

Creative Commons: Attribution-NonCommercial (CC BY-NC)

### Document Version

Publisher's PDF, also known as Version of record

### Citation for published version (Harvard):

Rauschenbach, M, Lawrenson, SB, Taresco, V, Pearce, AK & O'Reilly, RK 2020, 'Antimicrobial hyperbranched polymer-usnic acid complexes through a combined ROP-RAFT strategy', *Macromolecular Rapid Communications*. <https://doi.org/10.1002/marc.202000190>

[Link to publication on Research at Birmingham portal](#)

### General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.



# Antimicrobial Hyperbranched Polymer–Usnic Acid Complexes through a Combined ROP-RAFT Strategy

Moritz Rauschenbach, Stefan B. Lawrenson, Vincenzo Taresco, Amanda K. Pearce,\* and Rachel K. O'Reilly\*

**Polymer–drug conjugates have received considerable attention over the last decades due to their potential for improving the clinical outcomes for a range of diseases. It is of importance to develop methods for their preparation that have simple synthesis and purification requirements but maintain high therapeutic efficacy and utilize macromolecules that can be cleared via natural excretory pathways upon breakdown. Herein, the combination of ring-opening polymerization (ROP) and reversible addition–fragmentation chain-transfer (RAFT) polymerization is described for the straightforward synthesis of amphiphilic, stimuli-responsive, biodegradable, and highly functionalizable hyperbranched polymers. These unimolecular nanoparticles demonstrate a versatile platform for the synthesis of polymer–drug conjugates owing to the inclusion of a Boc-protected polycarbonate moiety in either a block or random copolymer formation. A proof-of-concept study on the complexation of the poorly water-soluble antimicrobial drug usnic acid results in polymer–drug complexes with powerful antimicrobial properties against gram-positive bacteria. Therefore, this work highlights the potential of amphiphilic and biodegradable hyperbranched polymers for antimicrobial applications.**

Antimicrobial resistance (AMR) is a global problem, with infections caused by multidrug-resistant bacteria now reaching critical levels. The need for new antimicrobial agents is currently a well-documented and important issue relating to world health.<sup>[1,2]</sup> The World Health Organisation (WHO) has identified 12 species of bacteria for which new innovations and effective antimicrobial agents without pre-existing resistance are

urgently needed.<sup>[3]</sup> Nanomedicine represents a promising therapeutic strategy for addressing this major problem in the clinic, particularly in combating drug resistance in infectious diseases. Synthetic antimicrobial polymers, such as amphiphilic and cationic polymers, have emerged as promising candidates for further development as antimicrobial agents with decreased potential for development of resistance.<sup>[4–6]</sup>

Synthetic polymers possess the powerful ability to be modified with multiple chemistries, active agents, and functionalities. They can be endowed with intrinsic antimicrobial activity by mimicking chemistries and functions of antimicrobial peptides, notably cationic moieties that interact with negatively charged bacterial cell walls, whilst hydrophobic counterparts can be used to facilitate membrane penetration or hydrophilic components to facilitate water-solubility in a drug delivery setting.<sup>[7]</sup> In

general, the cationic, amphiphilic polymers exhibit selectivity to bacterial targets through favorable electrostatic interactions between the polymer and the highly negatively charged bacterial cell surface.<sup>[5]</sup> The further self-assembly of amphiphilic polymers into nanoparticles can also increase efficacy over small molecule drugs and linear polymers by exploiting multivalency of functional groups to enable higher cell recognition and binding capabilities, improved penetration through the cell membrane, and ultimately, higher antimicrobial activity.<sup>[8]</sup> In isolation, antimicrobial polymers can induce bacterial cell death through mechanisms such as membrane destabilization and lysis, inhibition of energy metabolism, impaired respiratory function, and apoptotic-like cell death. Importantly, therapeutic nanoparticles can be further functionalized with small molecule drugs to limit bacterial expression of resistance by imparting multimodal mechanisms of action, by expressing multiple antimicrobial features that work synergistically.<sup>[9]</sup>

Usnic acid (UA) is a naturally occurring dibenzofuran derivative isolated from lichen, and demonstrates interesting biological properties, including powerful antimicrobial action.<sup>[10]</sup> Of particular relevance, it has been shown to strongly inhibit the growth of a range of planktonic gram-positive bacteria species, such as multi-resistant strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Mycobacterium tuberculosis*.<sup>[11,12]</sup> *S. aureus* is an opportunistic pathogen and a common cause of healthcare-associated infections, including

M. Rauschenbach, Dr. S. B. Lawrenson, Dr. A. K. Pearce,  
Prof. R. K. O'Reilly  
School of Chemistry  
University of Birmingham  
Edgbaston, Birmingham B15 2TT, UK  
E-mail: A.K.Pearce@bham.ac.uk; R.OReilly@bham.ac.uk

Dr. V. Taresco  
School of Chemistry  
The University of Nottingham  
University Park  
Nottingham NG7 2RD, UK

The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/marc.202000190>.

© 2020 The Authors. Published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

DOI: 10.1002/marc.202000190

skin infections (e.g., abscesses), respiratory infections (e.g., sinusitis), and food poisoning.<sup>[13]</sup> Significantly, *S. aureus* is one of the five most common causes of hospital-acquired infections and is often the cause of wound infections following surgery, and therefore it is crucial to develop new and effective strategies to combat this class of infection.<sup>[14]</sup> Unfortunately, despite its therapeutic potential, applications of UA have been limited by its poor solubility in water and its hepatotoxicity.<sup>[15,16]</sup>

Previously, we have demonstrated a facile and highly-accessible route to a range of amphiphilic polymeric materials through a combined ring-opening polymerization (ROP) and reversible addition–fragmentation chain-transfer (RAFT) polymerization strategy.<sup>[17,18]</sup> Particularly, we have shown that the use of 2-hydroxyethyl methacrylate (HEMA) as a bifunctional initiator for the ROP step gives ready access to biodegradable macromonomers that can be polymerized into interesting final architectures, such as hyperbranched polymers (HBs).<sup>[19]</sup> HBs show great promise for drug delivery applications on account of their unimolecular nature (no critical aggregation concentration), small particle sizes (less than 50 nm), and their highly branched structure, which provides a high local density of functional groups for further functionalization with drugs.<sup>[20,21]</sup> While linear, cationic homopolymers possess antimicrobial activity through disruption of the bacteria cell membrane, they have limited potential for codelivery of a second drug due to their high water-solubility and lack of additional chemical handles.<sup>[22]</sup> Formulation into polymeric nanoparticles can facilitate the inclusion and controlled delivery of small molecule drugs; however, the larger size and masking of charged groups can hinder their therapeutic performance.<sup>[23]</sup> On the contrary, the unique properties of HBs can combine the advantages of both strategies by ensuring accessibility and flexibility of the cationic groups in solution, while also providing a high density of functional groups for attachment of additional therapeutics.

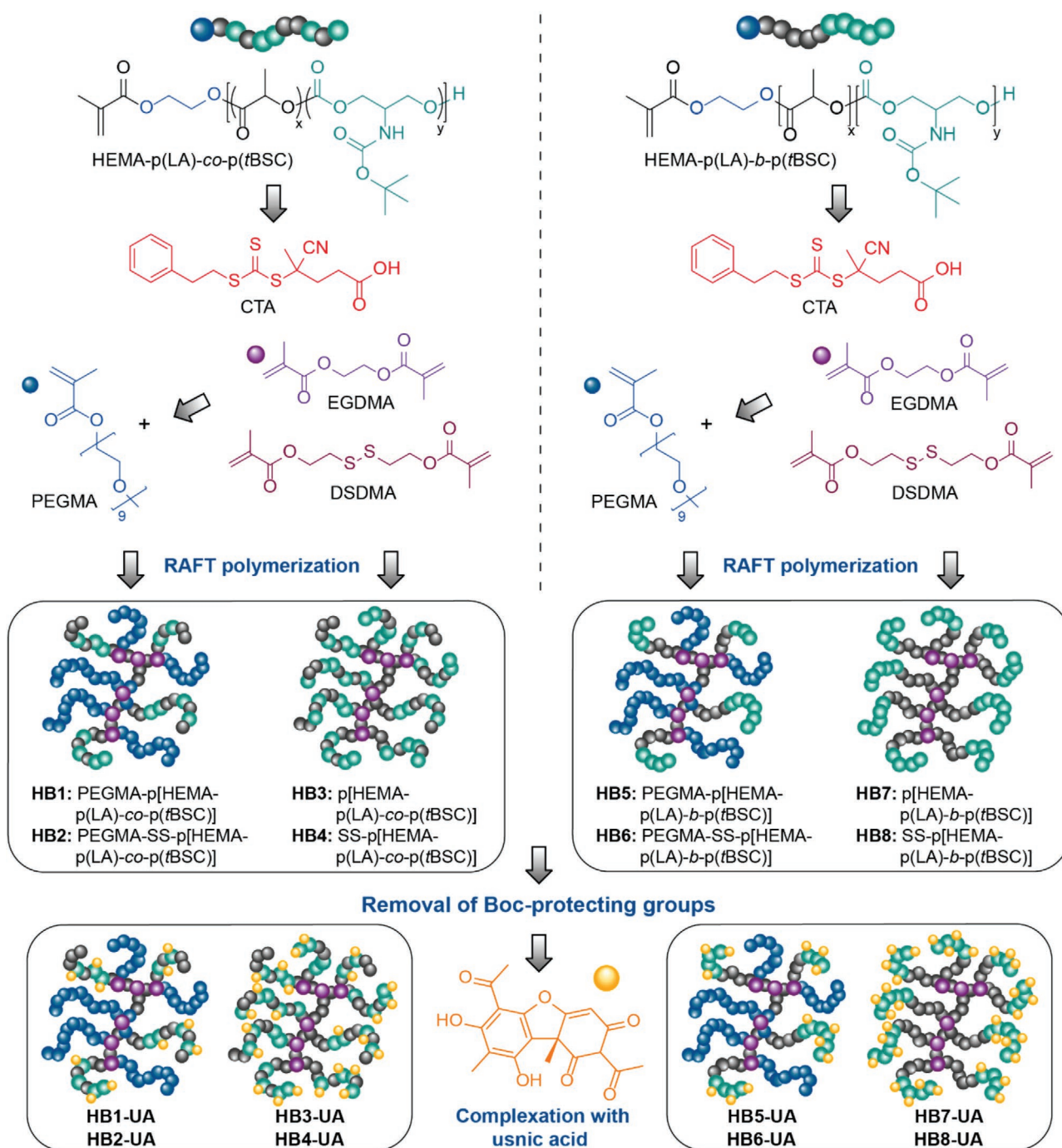
In previous work, we investigated the copolymerization of the pharmaceutically relevant lactide with a Boc-protected serinol-based cyclic carbonate monomer (*t*BSC) to produce a biodegradable material capable of further functionalization,<sup>[24,25]</sup> demonstrated through the conjugation of a model chemotherapeutic drug doxorubicin and a novel nitric oxide photodonor moiety.<sup>[19,26]</sup> However, given the presence of the charged amine groups following the trifluoroacetic acid (TFA) deprotection step, we envisaged that these polymers could also be exploited for antimicrobial applications in a simple fashion. Specifically, through further complexation of the polymer particles with UA, we can achieve in a single step a powerful combination approach that can both improve the apparent solubility and bioactivity of the drug, and demonstrate a simple method to form polymer–drug complexes without the need for additional excipients.<sup>[27–29]</sup>

Based on this, in this work, we sought to investigate the potential of amphiphilic and biodegradable hyperbranched polymers for antimicrobial applications in combination with the powerful drug usnic acid. We were further interested to explore the effect of monomer distribution on drug complexation and antimicrobial performance by comparing particles synthesized using macromonomers of random or block copolymerized lactide and *t*BSC. The hyperbranched polymers were synthesized incorporating a nonresponsive crosslinker, as well as

investigating the inclusion of a disulfide-responsive crosslinker, which we hypothesized to be of interest for future studies with successful conjugates due to the potential of redox-responsive groups to counteract biofilm formation and drug resistance triggered by the presence of endogenous oxidative reactions.<sup>[30,31]</sup> Lastly, the importance of water solubility of the final conjugates was explored by preparing two classes of HBs, comprising either 100 mol% of the biodegradable macromonomer or as a 10 mol% copolymer with poly(ethylene glycol) methyl ether methacrylate (PEGMA). The resultant polymer library was transformed into cationic particles through Boc-deprotection using TFA, with subsequent complexation with usnic acid and was ultimately evaluated for their antimicrobial activity against wild type and drug resistant mutant strains of *S. aureus*. The overall synthetic strategy is depicted in **Scheme 1**.

A ring-opening polymerization method initiated by 2-hydroxyethyl methacrylate was employed in order to produce hydrophobic and functionalizable macromonomers amendable to further radical polymerization reactions. First, the Boc-protected cyclic carbonate monomer (*t*BSC) was synthesized in two steps starting from serinol as previously described, and fully characterized to confirm that no free alcohol groups remained.<sup>[19]</sup> D,L-Lactide (LA) was used as a comonomer due to its ubiquitous presence in the drug delivery field and to tune the hydrophobicity and degradation rates of the final macromolecules. As shown in **Figure 1**, two different macromonomers were synthesized targeting 15 repeat units of both the LA and *t*BSC, as either a random copolymer or as an A–B block copolymer, in order to investigate the influence of functional group placement. The ROPs were performed at room temperature in anhydrous dichloromethane (DCM) with 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) as organocatalyst. Each ROP step was complete within 10 min, reaching >99% conversion. <sup>1</sup>H NMR spectroscopy confirmed final DP values in good agreement with feed ratios, indicating integrity of the ROP process (Figures S1 and S2, Supporting Information).

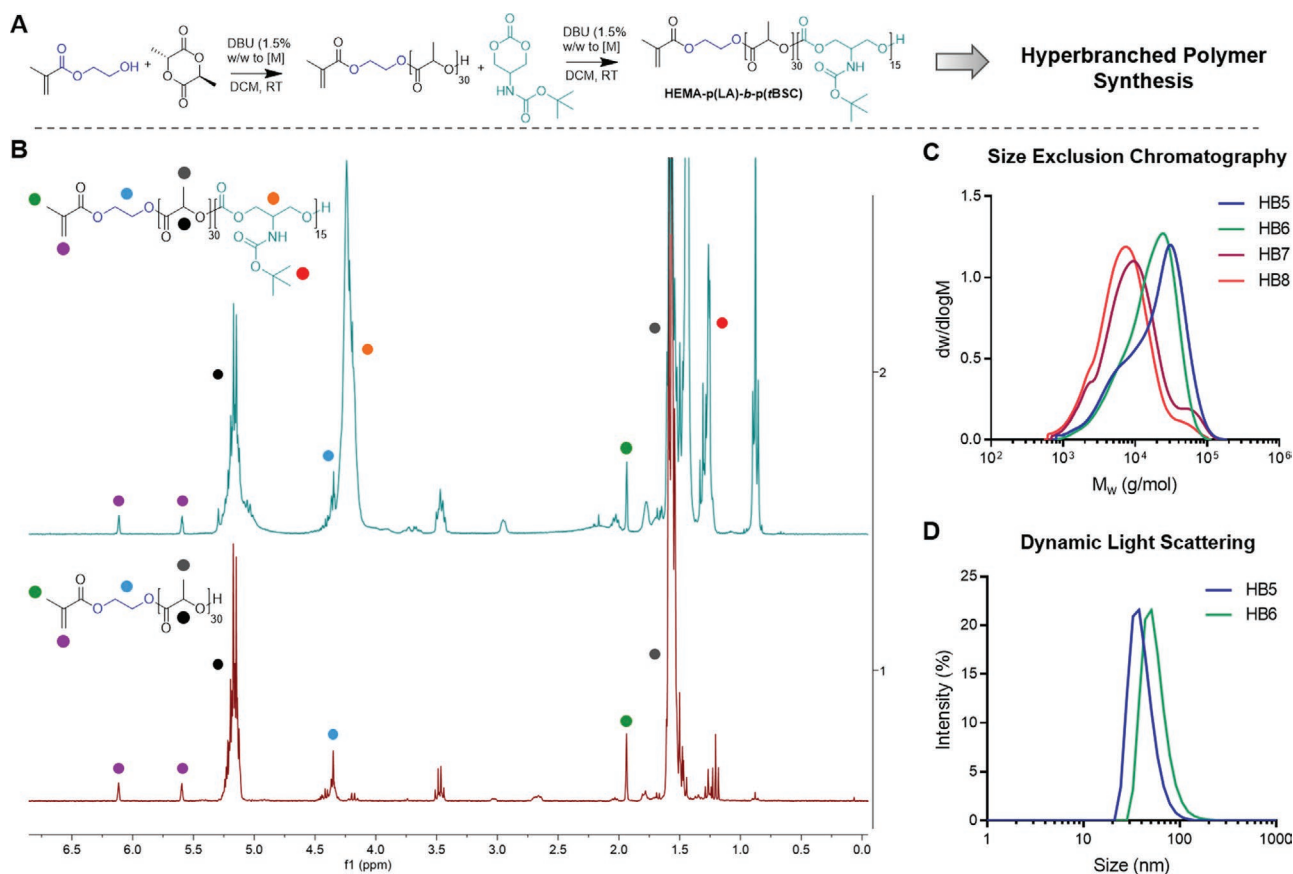
To produce nanoparticles, the two macromonomers, HEMA-p(LA)<sub>15-co-p</sub>(*t*BSC)<sub>15</sub> and HEMA-p(LA)<sub>15-b-p</sub>(*t*BSC)<sub>15</sub> were then further polymerized into hyperbranched polymer structures using RAFT polymerization. This process allows for the semi-controlled formation of dense, highly branched structures that form unimolecular nanoparticles in solution of sizes less than 100 nm without the need for surfactants or formulation steps. The addition of a low molar ratio (<10%) of a bis-methacrylate crosslinker promotes the formation of hyperbranched polymers, and in this work we were interested to investigate two different chemistries: the nondegradable and commercial ethylene glycol dimethacrylate (EGDMA) and a redox responsive disulfide-containing bismethacrylate (DSDMA). Initially, the ROP macromonomers are hydrophobic and therefore have no solubility in aqueous environments; however, following removal of the Boc-protecting groups with TFA, the charged amine groups should increase the solubility. Based on this, the final variable we were interested to investigate was the influence of water-solubility on both drug loading and antimicrobial performance. To achieve this, HBs were formed from either 100 mol% of the macromonomer or as a copolymer with 90 mol% PEGMA. In total eight HBs were synthesized combining the three variables, shown in Scheme 1 and **Table 1**.



**Scheme 1.** The overall strategy for the polymer–drug complexes synthesized in this work.

With the exception of the nature of the monomer feed and chemistry of the crosslinker, all other polymerization conditions were identical.  $^1\text{H}$  NMR spectroscopy and size-exclusion chromatography (SEC) analysis confirmed the successful attainment of eight unique hyperbranched polymer species (Figures S3–S10, Supporting Information). Due to the complex chemistry of the HBs resulting in multiple overlapping signals within the  $^1\text{H}$  NMR spectra, polymerization conversions were not able to be determined; however, almost

complete consumption of the vinyl peaks (6.1 and 5.6 ppm) confirmed that the polymerization had taken place. This was further confirmed through SEC analysis of the final macromolecules, which showed the presence of a relatively large molecular weight species, with broad dispersities that are typical of hyperbranched polymer architectures (Figure 1, Table 1). Dynamic light scattering (DLS) measurements were performed in 0.5 M NaCl for the soluble PEGMA copolymers which confirmed particles with sizes ranging from 35 to 68 nm.



**Figure 1.** A) Synthetic route towards the block copolymer macromonomer HEMA-p(LA)<sub>15</sub>-b-p(tBSC)<sub>15</sub>. B) Characterization of the purified products by <sup>1</sup>H NMR spectroscopy. C) Representative SEC traces for the block copolymer macromonomer HB series, and D) representative DLS data in 0.5 M NaCl for the water-soluble PEGMA copolymers.

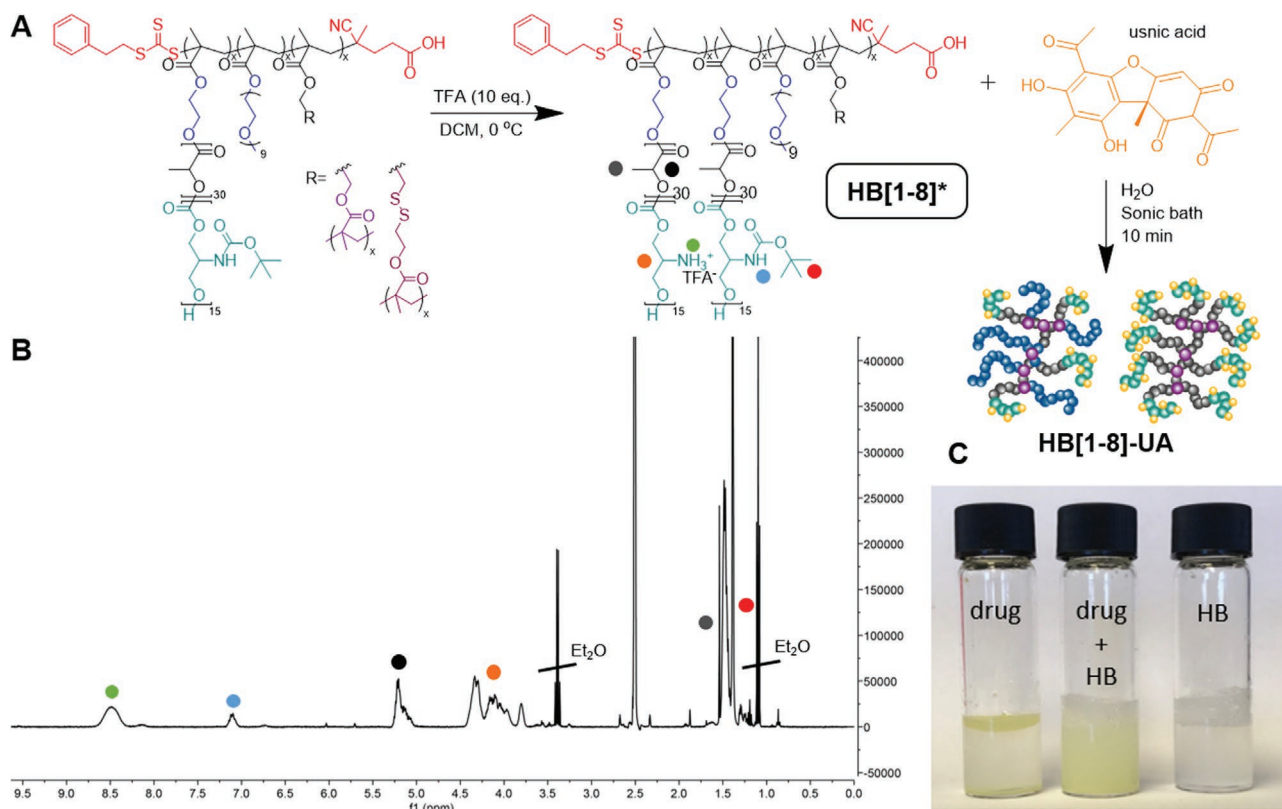
Following the successful synthesis of the base hyperbranched molecules, the Boc-protecting groups were removed to yield the free charged amine species (Figure 2). This reaction was performed as previously reported using TFA in anhydrous DCM at 0 °C to avoid degradation of the polyester/polycarbonate backbones.<sup>[19,26]</sup> The appearance of the ammonium peak by <sup>1</sup>H NMR spectroscopy confirmed the unmasking of on average 50% of the amine groups (Figures S11–S18, Supporting Information). SEC analysis after deprotection showed no changes in the retention time or intensity of the polymer peaks indicating that no

unwanted hydrolysis had occurred (Figure S19, Supporting Information). Finally, zeta-potential measurements were performed on the deprotected HBs synthesized with EGDMA as representative samples. The two copolymers with PEGMA showed positive zeta potential values dependent on the macromonomer structure, with the random macromonomer showing an overall neutral charge (likely due to the sporadic arrangement of ammonium groups) and the block macromonomer showing an increase to +13.3 mV. As expected, the HBs formed from 100 mol% macromonomer had a higher positive value due to the increased

**Table 1.** SEC and DLS characterization of HBs 1–8.

Sample	Structure of macromonomer	M <sub>n</sub> (SEC)	D <sub>M</sub> (M <sub>w</sub> /M <sub>n</sub> )	D <sub>h</sub> (DLS)
HB1: PEGMA-p[HEMA-p(LA)-co-p(tBSC)]	Random	37 kDa	2.3	50 nm
HB2: PEGMA-SS-p[HEMA-p(LA)-co-p(tBSC)]	Random	107 kDa	1.2	35 nm
HB3: p[HEMA-p(LA)-co-p(tBSC)]	Random	20 kDa	2.7	a)
HB4: SS-p[HEMA-p(LA)-co-p(tBSC)]	Random	200 kDa	1.8	a)
HB5: PEGMA-p[HEMA-p(LA)-b-p(tBSC)]	Block	32 kDa	1.3	44 nm
HB6: PEGMA-SS-p[HEMA-p(LA)-b-p(tBSC)]	Block	52 kDa	2.1	68 nm
HB7: p[HEMA-p(LA)-b-p(tBSC)]	Block	96 kDa	2.1	a)
HB8: SS-p[HEMA-p(LA)-b-p(tBSC)]	Block	83 kDa	1.6	a)

a) not water-soluble.



**Figure 2.** A) Removal of Boc-protecting groups using TFA, B)  $^1\text{H}$  NMR spectrum confirming the appearance of the ammonium peak at 8.5 ppm, and C) photograph of aqueous suspensions of drug alone, complexed drug and HB alone, highlighting the increase in drug solubility as a result of HB complexation.

number of amine groups, with a zeta potential of approximately 45 mV for both HBs (Table S1, Supporting Information).

Subsequently, the hydrophobic drug usnic acid was complexed to the charged HBs through electrostatic interactions (Figure 2). The polymers and drug were sonicated for 10 min in water to facilitate dispersal of the drug throughout the polymer matrix. The complexes were purified to remove un-complexed UA through centrifugation and the supernatant recovered by lyophilization. Quantification of UA complexation was performed using UV-vis analysis in dimethyl sulfoxide (DMSO) at 280 nm (Table 2). As expected, the HBs comprised of 100 mol% macromonomer had a greater drug loading than those of the copolymers with PEGMA as a result of a greater number of available charged amine groups. Interestingly, it could also be seen that the block macromonomer structure could facilitate both a higher drug content and encapsulation efficiency than the random structure. This can perhaps be attributed to the multiple charged groups on usnic acid requiring interaction with multiple charges on the HBs, and thus more efficacious interactions when in closer proximity.<sup>[27]</sup>

The complexes were evaluated for their antimicrobial activity in two strains of gram-positive *S. aureus*, wild type SA01 and drug resistant mutant SA02. The minimum inhibitory concentration (MIC) of the complexations was assessed through a broth microdilution assay, using the free drug (from 2% DMSO) and bare polymers as controls. After 24 h incubation, it can be seen that the complexes had a superior ability

to inhibit the growth of bacteria in comparison to the free drug (Table 2). This effect is particularly heightened for the 100 mol% macromonomer complexes, which is likely the result of the increased drug loading. Interestingly, the HBs comprised of the block copolymer structure, whether copolymerized with PEGMA or not, display greater antimicrobial performance than their random copolymer counterparts. For example, HB7-UA and HB8-UA have MIC values of 8 and 4 against SA01 and SA02 respectively, whereas HB3-UA and HB4-UA are increased to 31 and 16 respectively. Considering that the drug loading of these complexes were comparable ( $\approx 1:2$  HB:UA), this is potentially an effect of the higher density of charged groups and complexed drug towards the periphery of the HB particles, facilitating more rapid and complete drug release.<sup>[32,33]</sup>

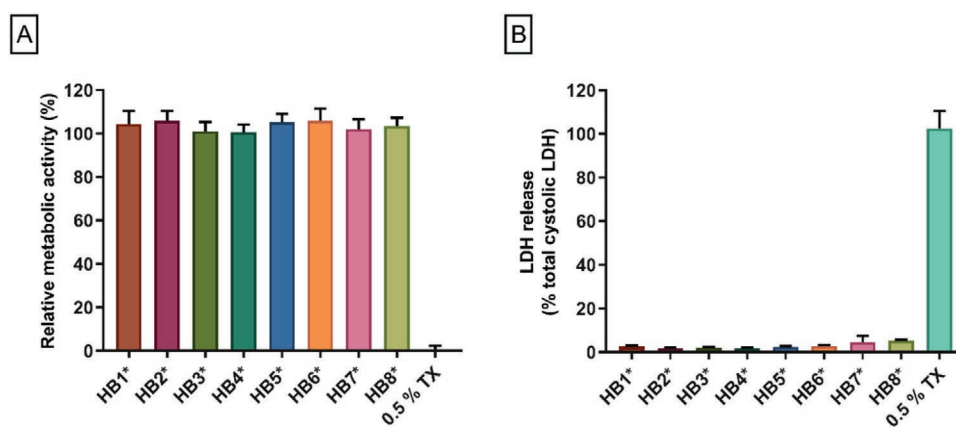
Finally, to verify the suitability of the complexes for potential in vivo drug delivery applications, the nondrug loaded (positively charged) HBs were assessed for their cytotoxicity and cell membrane integrity using A549 lung cancer cells as a model cell line. The cells were incubated with the bare polymer particles at a single fixed concentration of  $250 \mu\text{g mL}^{-1}$  (above the highest MIC value) for 48 h. It can be seen in Figure 3 that, despite the presence of charged amine groups, all eight HBs showed no signs of cytotoxicity in both assays, with no decrease in cellular metabolic activity or membrane disruption, as indicated by a lack of LDH release.<sup>[34]</sup>

Overall, the data in this study show that well-defined hyperbranched polymers can be produced exploiting versatile and

**Table 2.** Drug complexation characterization and antimicrobial evaluation.

Sample	Structure of macromonomer	Usnic acid complexation		MIC [ $\mu\text{g mL}^{-1}$ ]	
		Drug content (wt%)	Encapsulation efficiency (%EE)	SA01	SA02
Usnic acid <sup>a)</sup>	–	–	–	250	125
HB1-UA	Random	2.34	18.99	125	63
HB2-UA	Random	4.12	33.48	125	63
HB3-UA	Random	32.63	26.53	31	16
HB4-UA	Random	37.31	30.33	31	16
HB5-UA	Block	3.64	29.55	63	31
HB6-UA	Block	6.19	50.30	63	31
HB7-UA	Block	45.88	37.29	8	4
HB8-UA	Block	50.85	41.34	8	4

<sup>a)</sup>from DMSO.



**Figure 3.** Cytocompatibility of deprotected HBs on A549 (lung carcinoma) cells. Cytotoxicity was determined by A) PrestoBlue metabolic activity and B) LDH release as an indicator of membrane damage. Data are presented as mean  $\pm$  SD ( $n = 4$ ).

controllable synthetic routes, with few steps and minimal purifications. Therefore, this work has demonstrated a straightforward route to highly functionalized, biodegradable materials which show promise for a variety of therapeutic applications.

In conclusion, we describe the synthesis and evaluation of antimicrobial hyperbranched polymer complexes using a combined ROP and RAFT strategy. Unimolecular, highly branched nanoparticles were obtained featuring biodegradable and functionalizable monomeric repeat units, with the inclusion of a Boc-protected polycarbonate moiety to facilitate the complexation of the poorly water-soluble drug usnic acid through electrostatic interactions. The resultant polymer–drug complexes showed powerful antimicrobial activity towards two strains of *S. aureus*, dependent on the chemistry of the polymer particles and the arrangement of the charged amine groups in the polymer chains. Overall, HBs comprised from a block copolymer macromonomer structure showed the greatest drug delivery performance, likely as a result of the higher density of drug molecules on the periphery of the polymer particles. Despite the presence of charged amine groups, the bare polymer particles showed no cytotoxicity or cell membrane damage in a model cancer cell line, highlighting the suitability

of the HBs for in vivo biological applications. Therefore, this work has demonstrated a straightforward, versatile, and highly accessible route to the formation of polymer–drug complexes without the need for additional excipients.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

## Acknowledgements

The authors are grateful to Dr. Jessica Blair and Helen McNeil for access, expert advice, and training in microbiology experiments. The authors would like to thank the European Research Council for funding (Grant number 615142).

## Conflict of Interest

The authors declare no conflict of interest.

## Keywords

antimicrobials, drug complexation, hyperbranched polymers, nanoparticles, ring opening polymerization

Received: April 7, 2020  
Revised: April 27, 2020  
Published online:

- [1] P. Fernandes, *Nat. Biotechnol.* **2006**, *24*, 1497.  
[2] S. B. Levy, M. Bonnie, *Nat. Med.* **2004**, *10*, S122.  
[3] S. Amann, K. Neef, S. Kohl, *Eur. J. Hosp. Pharm.* **2019**, *26*, 175.  
[4] E. R. Kenawy, S. D. Worley, R. Broughton, *Biomacromolecules* **2007**, *8*, 1359.  
[5] E. F. Palermo, K. Kuroda, *Appl. Microbiol. Biotechnol.* **2010**, *87*, 1605.  
[6] G. J. Gabriel, A. Som, A. E. Madkour, T. Eren, G. N. Tew, *Mater. Sci. Eng., R* **2007**, *57*, 28.  
[7] K. Kuroda, G. A. Caputo, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.* **2013**, *5*, 49.  
[8] A. Gupta, S. Mumtaz, C. H. Li, I. Hussain, V. M. Rotello, *Chem. Soc. Rev.* **2019**, *48*, 415.  
[9] S. J. Lam, N. M. O'Brien-Simpson, N. Pantarat, A. Sulistio, E. H. H. Wong, Y. Y. Chen, J. C. Lenzo, J. A. Holden, A. Blencowe, E. C. Reynolds, G. G. Qiao, *Nat. Microbiol.* **2016**, *1*, 1.  
[10] M. Cocchietto, N. Skert, P. Nimis, G. Sava, *Naturwissenschaften* **2002**, *89*, 137.  
[11] M. Lauterwein, M. Oethinger, K. Belsner, T. Peters, R. Marre, *Antimicrob. Agents Chemother.* **1995**, *39*, 2541.  
[12] D. F. Ramos, P. E. A. Da Silva, *Pharm. Biol.* **2010**, *48*, 260.  
[13] S. Y. C. Tong, J. S. Davis, E. Eichenberger, T. L. Holland, V. G. Fowler, *Clin. Microbiol. Rev.* **2015**, *28*, 603.  
[14] M. N. Swartz, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 2420.  
[15] E. Erba, D. Pocar, L. M. Rossi, *Farmaco* **1998**, *53*, 718.  
[16] N. P. da Silva Santos, S. C. Nascimento, M. S. O. Wanderley, N. T. Pontes-Filho, J. F. da Silva, C. M. M. B. de Castro, E. C. Pereira, N. H. da Silva, N. K. Honda, N. S. Santos-Magalhães, *Eur. J. Pharm. Biopharm.* **2006**, *64*, 154.  
[17] L. A. Ruiz-Cantu, A. K. Pearce, L. Burroughs, T. M. Bennett, C. E. Vasey, R. Wildman, D. J. Irvine, C. Alexander, V. Taresco, *Macromol. Chem. Phys.* **2019**, *220*, 1800459.  
[18] A. K. Pearce, C. E. Vasey, A. B. Anane-Adjei, F. Sodano, V. C. Crucitti, D. J. Irvine, S. M. Howdle, C. Alexander, V. Taresco, *Macromol. Chem. Phys.* **2019**, *220*, 1900270.  
[19] C. E. Vasey, A. K. Pearce, F. Sodano, R. Cavanagh, T. Abelha, V. Cuzzucoli Crucitti, A. B. Anane-Adjei, M. Ashford, P. Gellert, V. Taresco, C. Alexander, *Biomater. Sci.* **2019**, *7*, 3832.  
[20] A. K. Pearce, B. E. Rolfe, P. J. Russell, B. W.-C. B. W. C. Tse, A. K. Whittaker, A. V Fuchs, K. J. Thurecht, *Polym. Chem.* **2014**, *5*, 6932.  
[21] A. K. Pearce, J. D. Simpson, N. L. Fletcher, Z. H. Houston, A. V Fuchs, P. J. Russell, A. K. Whittaker, K. J. Thurecht, *Biomaterials* **2017**, *141*, 330.  
[22] S. K. Samal, M. Dash, S. Van Vlierberghe, D. L. Kaplan, E. Chiellini, C. Van Blitterswijk, L. Moroni, P. Dubruel, *Chem. Soc. Rev.* **2012**, *41*, 7147.  
[23] A. M. Carmona-Ribeiro, L. D. de Melo Carrasco, *Int. J. Mol. Sci.* **2013**, *14*, 9906.  
[24] W. Chen, F. Meng, R. Cheng, C. Deng, J. Feijen, Z. Zhong, *J. Controlled Release* **2014**, *190*, 398.  
[25] R. P. Brannigan, A. P. Dove, *Biomater. Sci.* **2017**, *5*, 9.  
[26] F. Sodano, R. J. Cavanagh, A. K. Pearce, L. Lazzarato, B. Rolando, A. Fraix, T. F. Abelha, C. E. Vasey, C. Alexander, V. Taresco, S. Sortino, *Biomater. Sci.* **2020**, *8*, 1329.  
[27] I. Francolini, V. Taresco, F. Crisante, A. Martinelli, L. D'Ilario, A. Piozzi, *Int. J. Mol. Sci.* **2013**, *14*, 7356.  
[28] V. Taresco, I. Francolini, F. Padella, M. Bellusci, A. Boni, C. Innocenti, A. Martinelli, L. D'Ilario, A. Piozzi, *Mater. Sci. Eng., C* **2015**, *52*, 72.  
[29] A. Martinelli, A. Bakry, L. D'Ilario, I. Francolini, A. Piozzi, V. Taresco, *Eur. J. Pharm. Biopharm.* **2014**, *88*, 415.  
[30] B. R. Boles, P. K. Singh, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 12503.  
[31] D. Ritz, J. Beckwith, *Annu. Rev. Microbiol.* **2001**, *55*, 21.  
[32] Y. Oda, K. Yasuhara, S. Kanaoka, T. Sato, S. Aoshima, K. Kuroda, *Polymers* **2018**, *10*, 93.  
[33] Y. Oda, S. Kanaoka, T. Sato, S. Aoshima, K. Kuroda, *Biomacromolecules* **2011**, *12*, 3581.  
[34] A. C. Engler, J. P. K. Tan, Z. Y. Ong, D. J. Coady, V. W. L. Ng, Y. Y. Yang, J. L. Hedrick, *Biomacromolecules* **2013**, *14*, 4331.