Antimicrobial Lactam Monomer and Polymer Sequestrants

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"Studies have demonstrated that commercially available antimicrobials and wound dressings are often ineffective in managing infections, owing to biofilms. Consequently, the development of smart and novel antibiofilm agents represents an area of growing importance in wound care, but also in medicine generally."

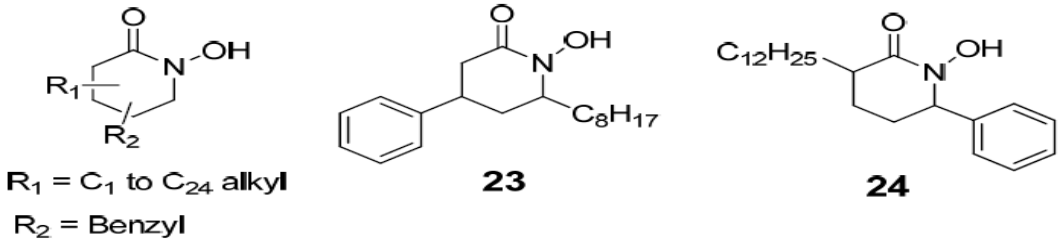
Percival, S. L. (2017). Importance of biofilm formation in surgical infection. *British Journal of Surgery*, *104*(2).

One way to kill pathogens even in biofilms is to deny them iron. Bacteria and fungus go to great lengths to steal iron from their hosts. Mammals try to reduce iron at infections but are not always successful. Why not give their defenses a boost with powerful polymeric sequestrants? Not only ferric ions but also zinc removal would be very significant.

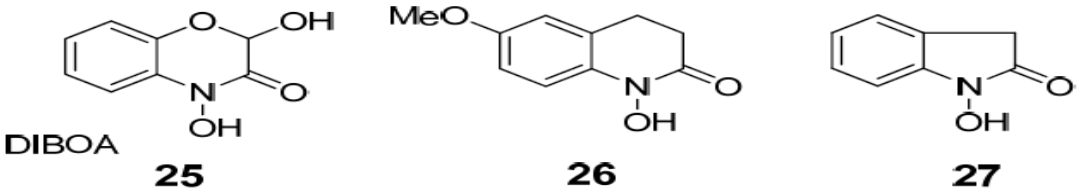
El-Gendy, N., Qian, J., Eshelman, K., Rivera, M., & Berkland, C. (2009). Antibiotic Activity of Iron-Sequestering Polymers. *Institute for Safe Medical Practices in*, *15*, 17.

Hydroxamic Acids

1-hydroxylactam-2-ones are important chelating compounds for a variety of medical applications. The following charts show several examples. I wandered how such compounds could be delivered to biological targets by polymeric systems. This led me to consider lactam monomers based on hydoxamic acids and other derivatives especially for wound care.



Scheme 10. Lipoxygenase inhibitors



Scheme 11. Prostate Cancer Drug Candidates

Banerjee, Ranjan.*Development of synthetic methodologies towards cyclic hydroxamic acid-based natural products*. Diss. Wake Forest University, 2010.

Although the above do not show 1-hydroxypyrrolin-2-one, it is considered a very important “privileged compound”. Since I like to think of ideas based on the very valuable and safe pyrrolidone lactam; therefore, this proposal is centered on them (but not exclusively as lactams in general can be substituted in most cases). The following chart illustrates the various routes to them in a recent review of the subject.

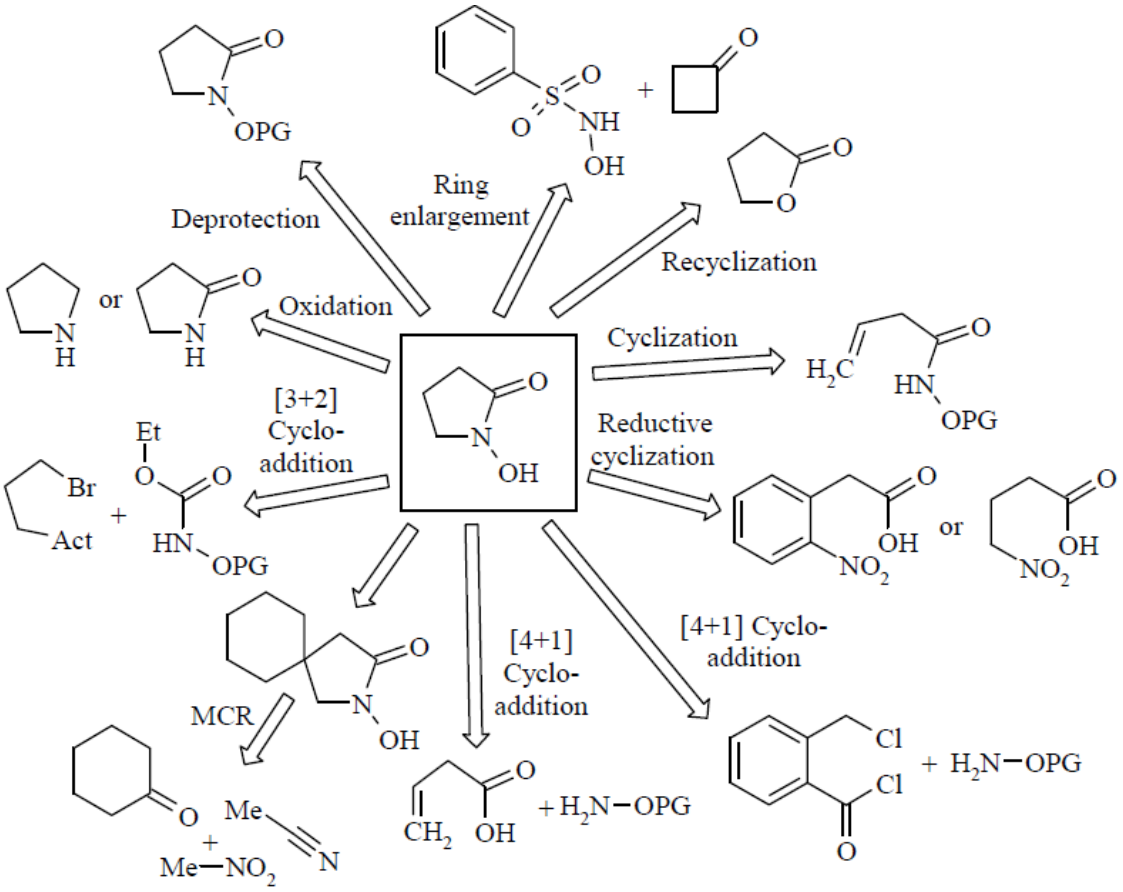


Fig. 4. Synthetic routes to 5-membered cyclic hydroxamic acids (5-CHA).

Trapencieris, P., J. Strazdina, and P. Bertrand. "Synthesis of small and medium size monocyclic hydroxamic acids (Review)."*Chemistry of Heterocyclic Compounds”,* 48.6 (2012): 833-855.

“What explains researchers' interest in CHA(cyclic hydroxamic acids)? Almost all of the ~300 Zn-dependent proteases now are known to be connected with tetrahedral Zn atom in the enzyme active center. The play for medicinal chemists in the development of new inhibitors for Zn-dependent enzymes is around the existing Zn-binding groups (ZBG). Despite a large number of publications in this field, research on new successful ZBGs is very limited. During the last decade, Cohen's group in USA has investigated several new ZBGs, among them

cyclic hydroxamic and cyclic thiohydroxamic acids. Therefore, we aimed to analyze all the synthetic routes to these perspective compounds."

Not only zinc based enzymes but iron itself are critical to bacteria:

US 8,946,188 B2

Aryl-capped iron-chelating siderophores assist various pathogens in acquiring iron inside their mammalian host, where iron is tightly chelated. The siderophores are essential for infection. In particular, siderophores are essential for infection by *Mycobacterium tuberculosis*, the causative agent for tuberculosis (de Voss et al. *Proc. Natl., Acad. Sci. USA* 97:1252-57, 2000; incorporated herein by reference), and *Yersinia pestis*, the etiological agent of the plague (de Almeid et al. *Microb. Pathog.* 14:9-21, 1993; Bearden et al. *Infect. Immun.* 65:1659-1668, 1997; each of which is incorporated herein by reference). Other pathogens which depend on siderophore-based iron acquisition systems include *Yersinia enterolitica, Pseudomonas aeruginosa, Bacillus anthracis, Vibrio vulnificus, Yersinia ruckeri, Brucella abortus, Burkholderia cepacia, Burkholderia cenocepacia, Bordetella bronchiseptica, Acinebacter calcoaceticus, Escherichia coli, Salmonella enterica, Shigella* spp., and *Vibrio cholerae* (Litwin et al., *Infect. Immun.* 64:2834-38, 1996; Bellaire et al., *Infect. Immun.* 71:1794-803, 2003; Boschiroli et al., *Curr. Opin. Microbiol.* 4:58-64, 2001; Sokol et al., *Infect. Immun.* 67:4443-55, 1999; Register et al., *Infect. Immun.* 69:2137-43, 2001; each of which is incorporated herein by reference). Inside their hosts, iron is relatively abundant but is tightly bound to intracellular and extracellular components (Weinberg, *Perspect. Biol. Med.* 36:215-221, 1993; incorporated herein by reference). The pathogenic bacteria synthesize siderophores to acquire Fe(III) from their hosts (Wooldridge and Williams, *FEMS Microbiol. Rev.* 12:325-348, 1993; incorporated herein by reference). Siderophore biosynthesis is, therefore, an attractive target for the development of new antibiotics to treat tuberculosis, plague, and other infection caused by microorganisms that depend on siderophore (e.g. *Pseudomonas aeruginosa*).

Saha, Ratul, et al. "Microbial siderophores: a mini review."*Journal of basic microbiology",* 53.4 (2013): 303-317.

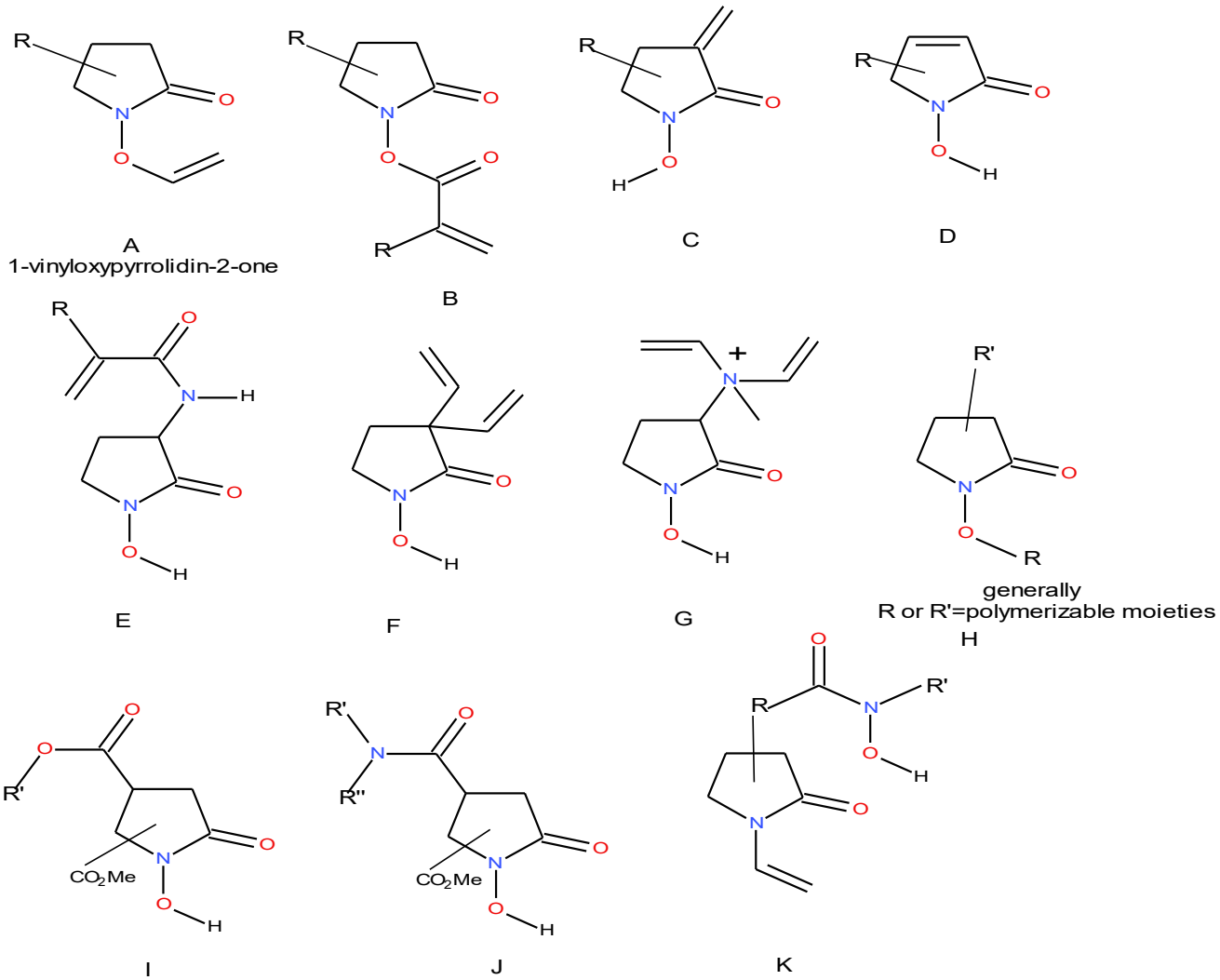
Shanzer, Abraham, Clifford E. Felder, and Yaniv Barda. "Natural and biomimetic hydroxamic acid based siderophores."*Patai's Chemistry of Functional Groups",* (2009).

Obviously one way around wound infection is to take the iron source away from the microbe with a polymeric sequestrant strong enough to permanently remove ferric ion. This was not the approach in the above patent where they are attacking the biosynthesis of the siderophile itself. My idea would supplement or supplant this patented approach, also killing the pathogen. Bacteria require ferric ion which is very insoluble and besides

it is held tightly by the infected host. Bacteria use siderophores to steal ferric ions and have figured out how to get the iron through the biofilm to where it is needed.

Several bacterial siderophores are based on hydroxamatic acid derivatives suggesting that polymeric types might out-gun them by preferentially grabbing iron(ferric ions). A polymeric motif should have even stronger complexation vs. the monomeric siderophores. Would injecting a powerful polymeric sequestrant into a wound weaken or kill invading bacteria?

Several potential hydroxamic acid pyrrolidone monomers and polymers can be visualized:



Scheme 1

Monomers A & B would require that the hydroxamic acid functionality be released from the polymer backbone and although they are interesting monomers, they don't fit the idea that hydroxamic acids in a polymer motif would be anti-siderophoric. Monomers C-

K would present the hydroxamic acid pendent to the polymer backbone or as part of the backbone in condensation based polymers and they would be worth considering.

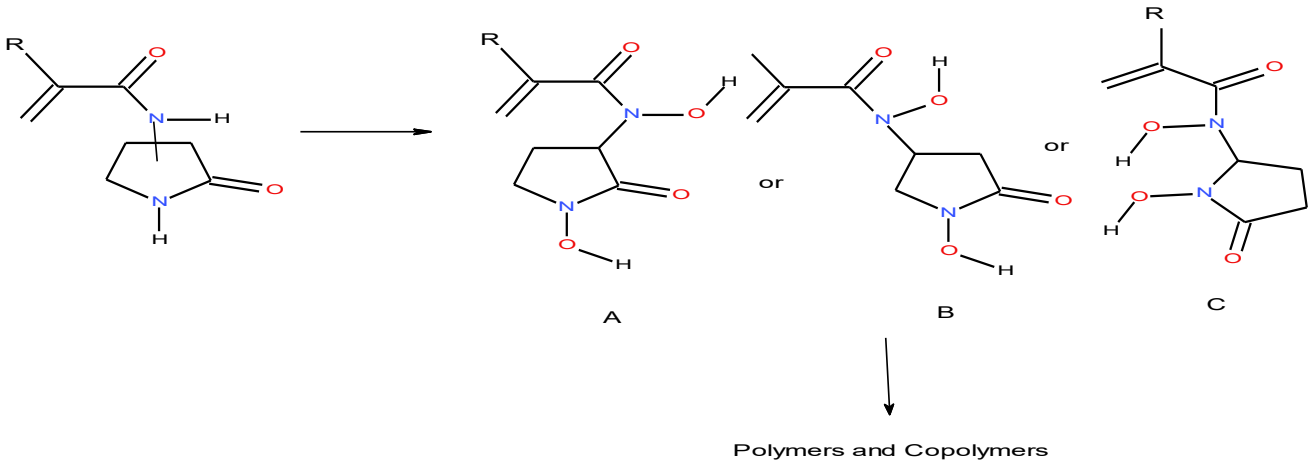
There are many examples already of polymeric hydroxamic acids employed as heavy metal ion sequestrants and zinc metalloproteinases suppressants.

Domb, Abraham J., et al. "Method of making hydroxamic acid polymers from primary amide polymers." U.S. Patent No. 5,128,420. 7 Jul. 1992.

Sefton, Michael, et al. "Hydroxyamate-containing materials for the inhibition of matrix metalloproteinases." U.S.Patent Application No. 11/714,730. US 2007/0160655 A1

Stopek, Joshua, and Ahmad Hadba. "Hydroxamate compositions." U.S. Patent No. 7,923,439. 12 Apr. 2011.

Possibly, chelatable polymeric hydroxamic acids are needed because they would be even stronger sequestrants! I'm suggesting bis-hydroxamic acrylamide monomers are possible:



Scheme 2

Because of the two hydroxamic moieties, one would expect stronger complexation and when polymerized, very strong complexation would be expected. This is because the hydroxamic oxygens are in a position to complex iron in a six membered ring. This is especially apparent with structure C. Block copolymerization by CRP(controlled radical polymerization) with NVP would afford strong complexation with the potential mildness and safety of PVP. Copolymers with PVP-iodine could result in an antiseptic with a dual kill mechanism.

The goal is to irreversibly remove iron and zinc ions from the infection or potential infection. Dangerous pathogens can form biofilms to protect themselves from antibiotics but they must have these heavy metal ions to live and thrive. Since most wounds heal

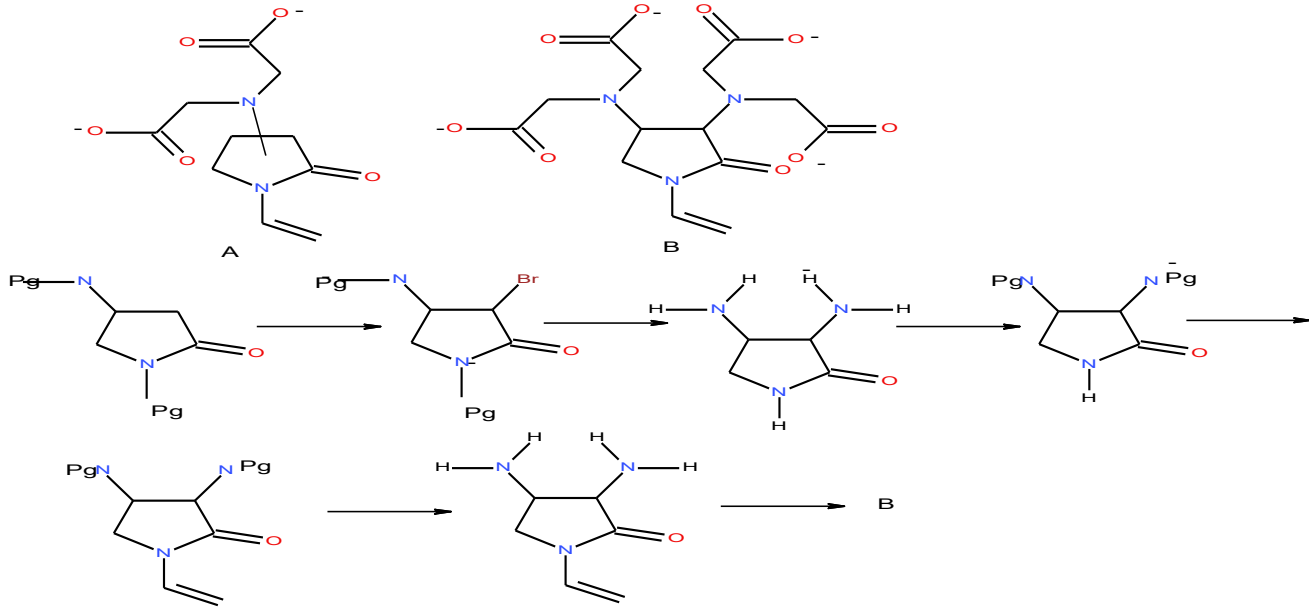
from the bottom up, removable polymeric sequestrants might be another method to prevent infection?

Said hydroxamic acid containing monomers can be copolymerized in various ways with NVP to produce polymers that are either crosslinked with polyfunctional comonomers or post crosslinked with actinic radiation, for example. Depending on the level of crosslinking, hard beads to loose gels can be prepared. Such crosslinked copolymers could be packed into a wound to sequester ferric and or zinc ions affording a very poor environment for bacteria. After use, they would be easily removed.

EDTA

Other references actually show how EDTA will starve bacteria for iron even successfully attacking them in biofilms.

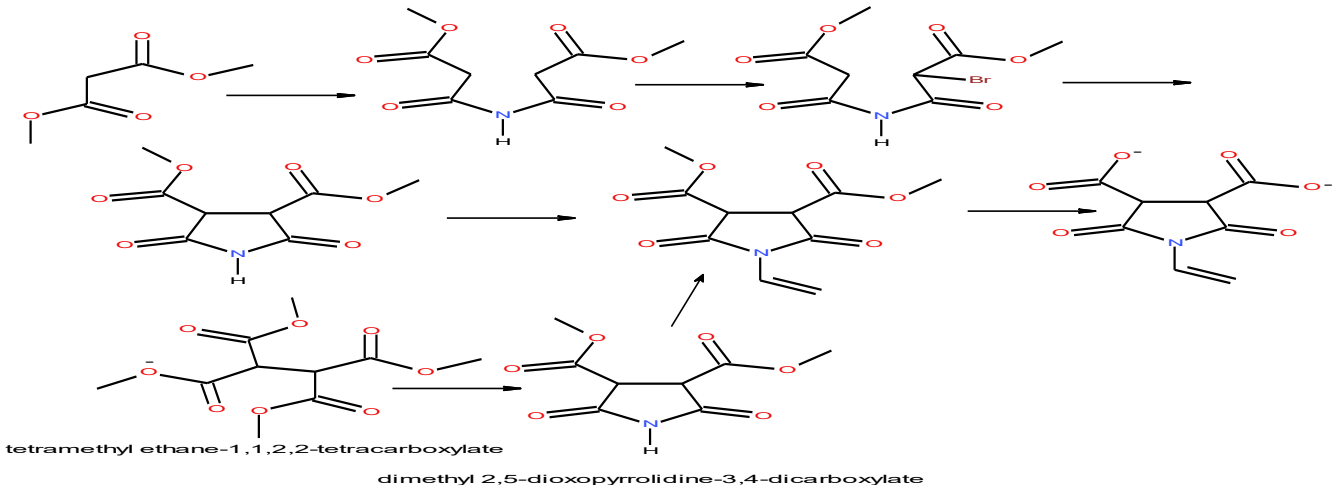
Therefor, I'm suggesting approaches to EDTA pyrrolidones:



Scheme 3: Compound “A” should be the easier to synthesize; however, a possible synthesis of “B" is proposed.

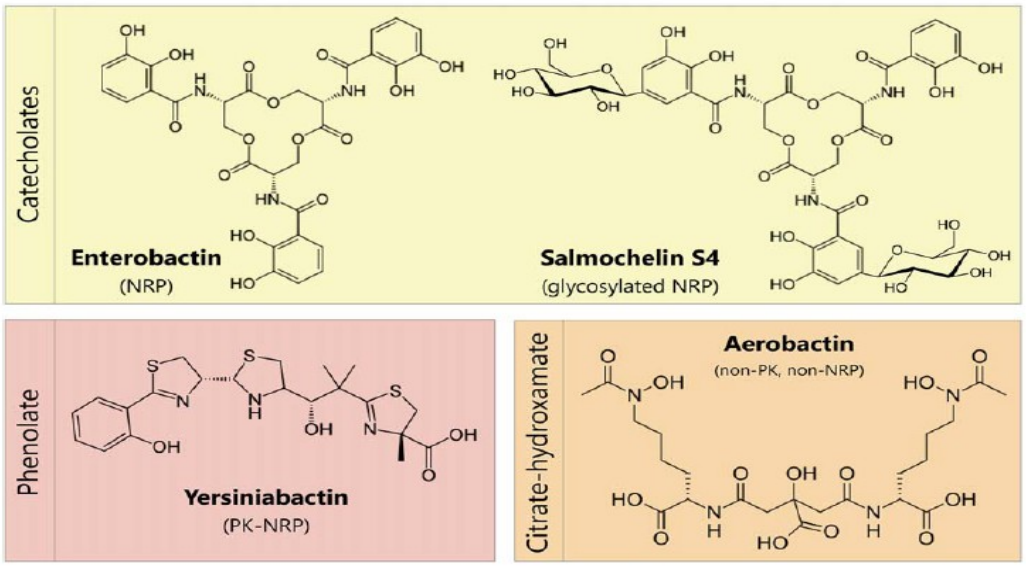
Finnegan, S., & Percival, S. L. (2015). EDTA: an antimicrobial and antibiofilm agent for use in wound care. *Advances in wound care*, *4*(7), 415-421.

Percival, S. L., Finnegan, S., Donelli, G., Vuotto, C., Rimmer, S., & Lipsky, B. A. (2016). Antiseptics for treating infected wounds: efficacy on biofilms and effect of pH. *Critical reviews in microbiology*, *42*(2), 293-309.



Scheme 4: Although not pyrrolidone derivatives, these imides look very doable. They are examples of carboxylate sequestrants.

Other siderophore structure types are known:

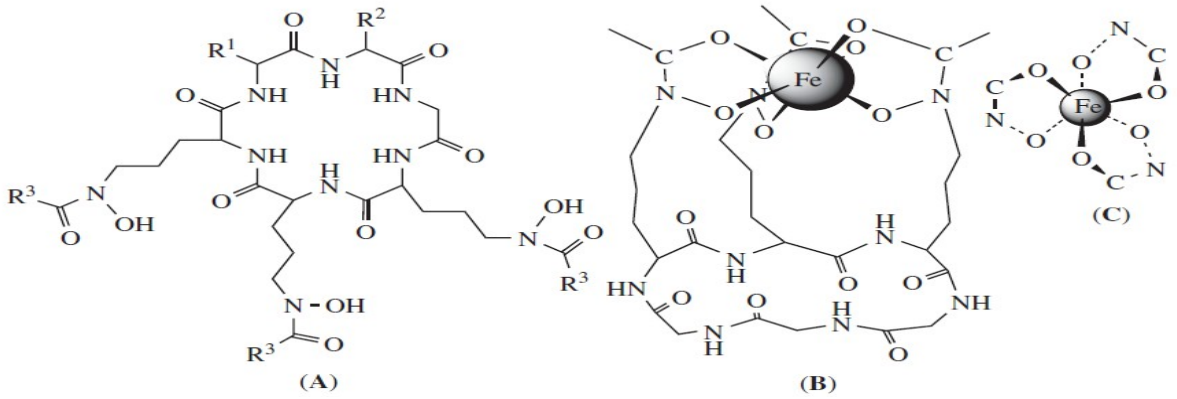


Structure of the siderophores synthesized by E. coli *(according to* 26*).*

Martin, Patricia, et al. "Interplay between siderophores and colibactin genotoxin biosynthetic pathways in Escherichia coli."*PLoS Pathog",* 9.7 (2013): e1003437.

The above siderophores complex ferric ion in a basket like structure where the chelation can use all three sequestrants in each in an octahedral arrangement.

TABLE 1. Natural ferrichromes and their structural variations [a]



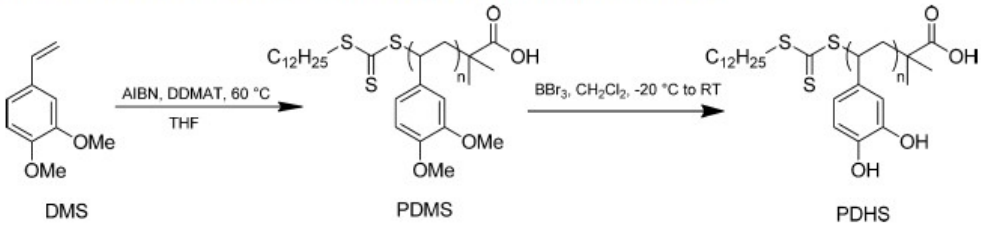
Shanzer, Abraham, Clifford E. Felder, and Yaniv Barda. "Natural and biomimetic hydroxamic acid based siderophores."*Patai's Chemistry of Functional Groups",* (2009).

When comparing Enterobactin and Salmochelin S4, addition of the sugar makes the latter too big for the host defense enzymes. Such modified siderophors are referred to as stealth siderophores because mammalian defense anti-siderophore proteins don't recognize them as siderophores, illustrating how powerful bacteria evolution can be, eventually overcoming whatever is placed against them. With the appearance of resistant pathogens especially in hospitals, requires new approaches for their elimination!

Stewart, P. S. (2015). Prospects for anti-biofilm pharmaceuticals. *Pharmaceuticals*, *8*(3), 504-511.

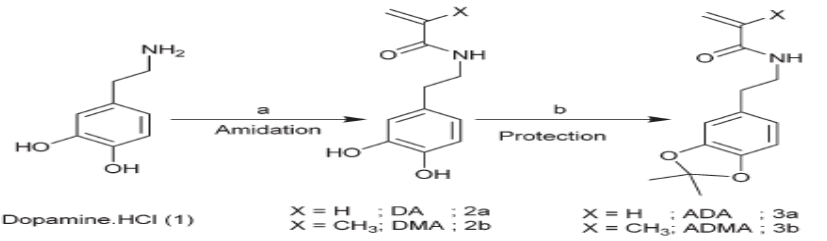
Catechols

Scheme 1. Synthetic Route for the Preparation of Poly(3,4-dihydroxystyrene), PDHS

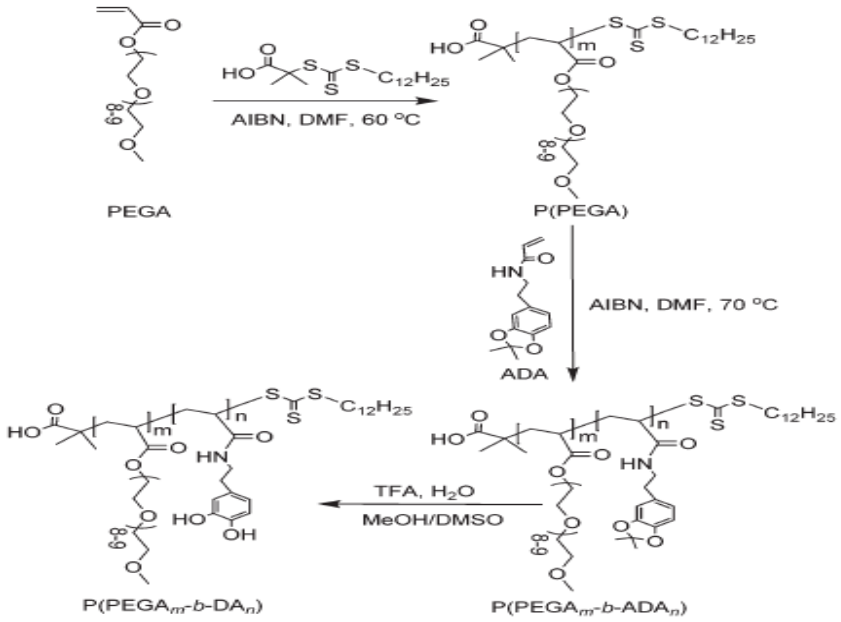


Enterobactin employs catechols to complex ferric ions; therefore, several catechol monomers can be prepared. In fact there is a growing literature concerning this subject.

Isakova, Anna, Paul D. Topham, and Andrew J. Sutherland. "Controlled RAFT polymerization and Zinc binding performance of catechol-inspired homopolymers."*Macromolecules",* 47.8 (2014): 2561-2568.

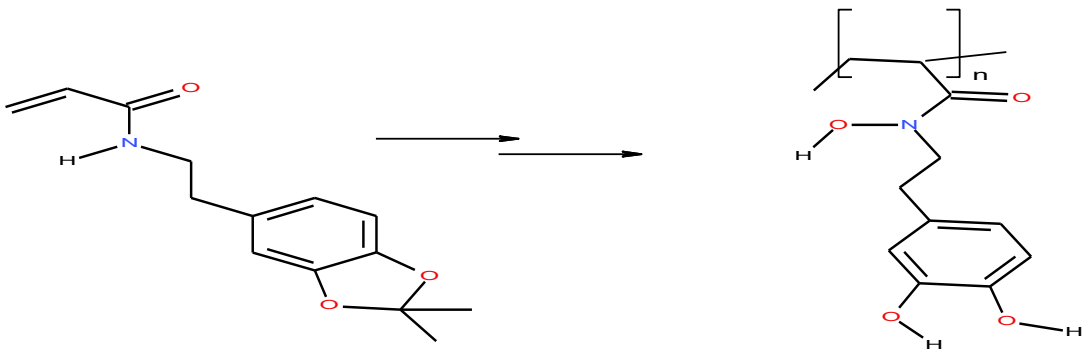


Scheme 2 Synthesis of unprotected (DA (**2a**), DMA (**3a**)), and acetonide-protected (ADA (**2b**), ADMA (**3b**)) monomers. Reagents and conditions: (a) **2a**: $Na_2B_4O_7{\cdot}10H_2O$, Na_2CO_3, H_2O (pH > 9), acryloyl chloride, 12 h, 85%; **2b**: $Na_2B_4O_7{\cdot}10H_2O$, Na_2CO_3, H_2O (pH > 9), methacrylic anhydride, 12 h, 81%; (b) *p*-TsOH, 2,2-dimethoxypropane, toluene, 2−4 h, **3a**: 90%, **3b**: 85%.

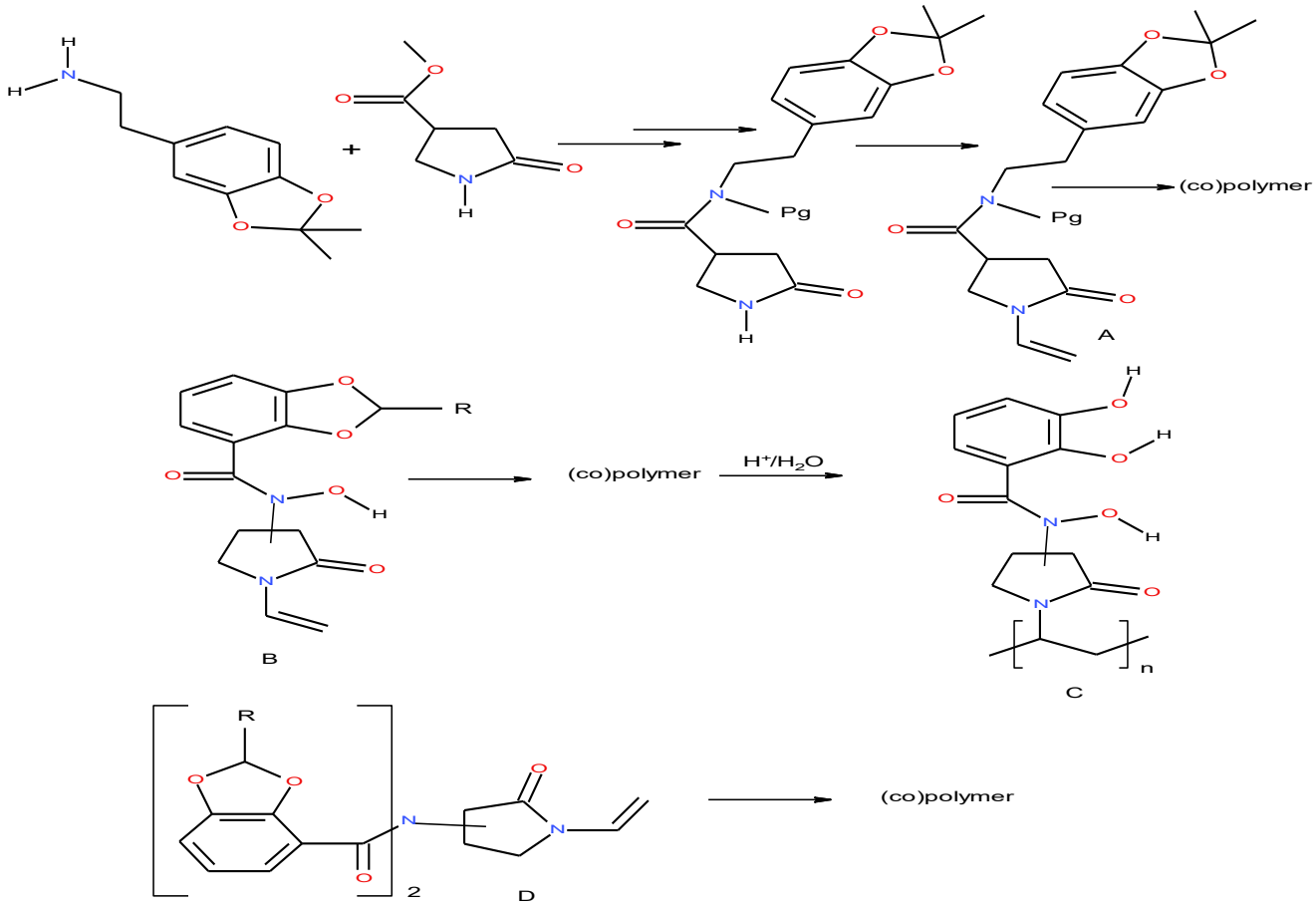


Scheme 3 Synthesis of P($PEGA_m$-*b*-ADA_n) copolymers through chain-extension of P(PEGA) macro-CTA with ADA and subsequent deprotection to afford free-catechol bearing P($PEGA_m$-*b*-DA_n).

Patil, N., Falentin-Daudré, C., Jérôme, C., & Detrembleur, C. (2015). Mussel-inspired protein-repelling ambivalent block copolymers: controlled synthesis and characterization. *Polymer Chemistry*, *6*(15), 2919-2933.



Scheme 5: Shows the ADA polymer or copolymer oxidized to the hydroxamic acid. This is my suggestion to increase the polymers ability to sequester and hold ferric ion away from pathogens.

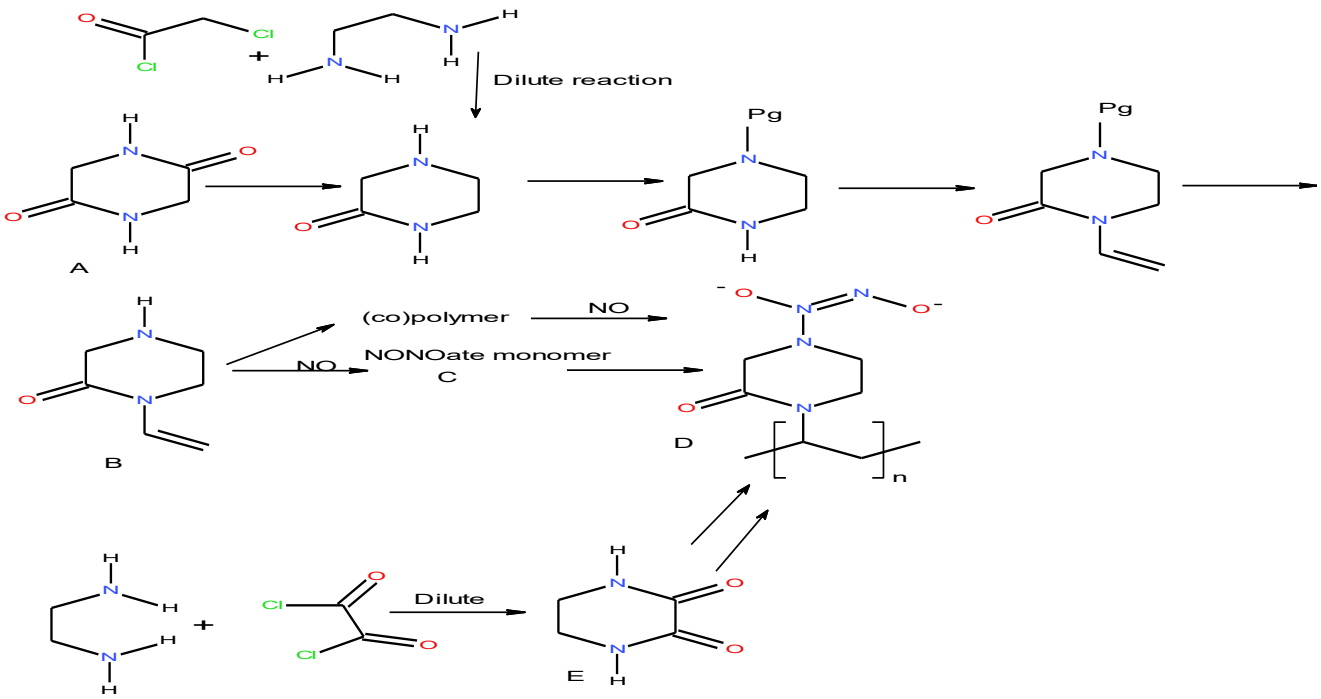


Scheme 6: “A” above could be oxidized to the hydroxamic acid pre-or post polymerization.

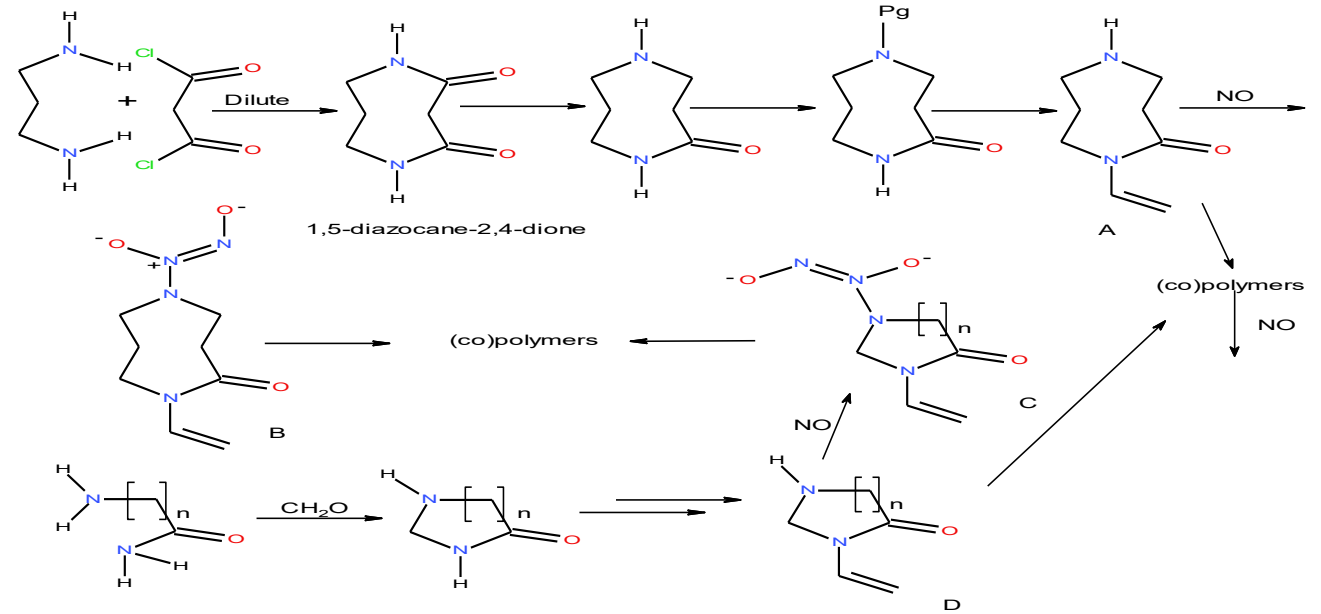
The catechol could obviously be part of other monomers that are FR polymerizable or copolymerizable with NVP, but NVP derivatives are shown above because they would be readily copolymerized with NVP itself. The acetal is a protective group that is base stable and would stand up to base catalyzed vinylation.

All of the above polymers would be film formers that could be designed to form insoluble crosslinked coatings on a wide variety of medically important implantable devices. PVP is safe enough to be thrombosis resistant. Copolymers of NVP with all the above said monomers would be expected to be candidates for these anti-biofilm coating applications! However, there is another design possibility to further enhance this application, nitric oxide release comonomers.

I don't know if NONOates interfere with FR polymerization; therefore, if that's a problem, monomer B can be polymerized and the polymer post reacted with NO.



Scheme 7: Since NO is a free radical, it might be necessary to first polymerize then NO.

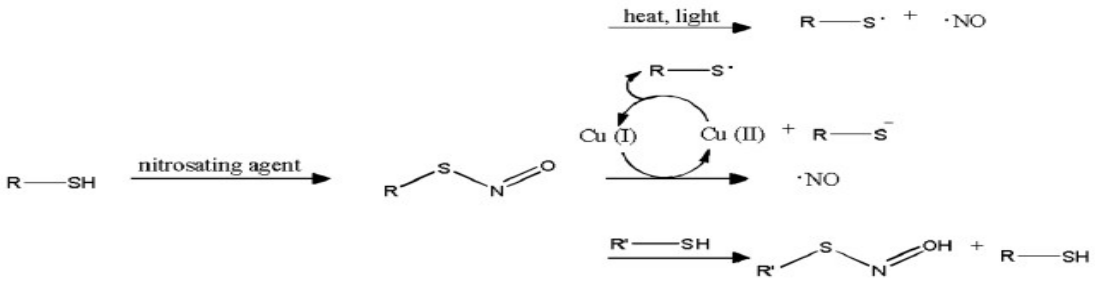


Scheme 8: A or D can individually be comonomers that are reacted with NO after polymerization. B and C, if the NONOate doesn't interfere, can be individually copolymerized.

Schemes 7 & 8 shows potential synthesis of what I think would be easier to obtain pyrrolidone like NONOates.

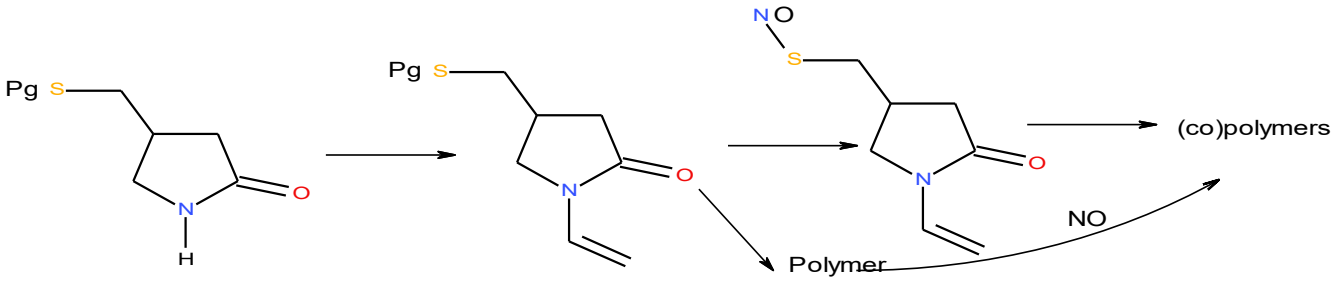
RSNO NO release agents.

"Based on their ability to spontaneously release NO in physiological media, NONOates and RSNOs represent the most widely used NO donor systems."



Scheme 2.
S-Nitrosothiol formation and decomposition.

Riccio, D. A., & Schoenfisch, M. H. (2012). Nitric oxide release: Part I. Macromolecular scaffolds. *Chemical Society Reviews*, *41*(10), 3731-3741.



Scheme 9:

This is one of many possible RSNO derivatives of NVP. I don't know if RSNO would interfere with FR polymerization so the reason for the post polymerization option for the NO reaction. I would hope that the RSNO monomer itself would be viable as it would be much easier to use in CRP.

NO release polymers are an important area of current research.

Wo, Yaqi, et al. "Recent advances in thromboresistant and antimicrobial polymers for biomedical applications: just say yes to nitric oxide (NO)."*Biomaterials science",* 4.8 (2016): 1161-1183.

In conclusion, I have suggested several NVP sequestrant derivatives that can be copolymerized with NVP or other monomers by various FR polymerization techniques. These suggested different monomer sequestrants can be combined to sequester ferric and zinc ions in more than one way, hopefully preventing bacterial biofilms from surviving in wounds and as anti-biofilm coatings on implants.

Huang, Keng-Shiang, et al. "Recent Advances in Antimicrobial Polymers: A Mini-Review."*International journal of*

molecular sciences", 17.9 (2016): 1578.

Thank you for reading this proposal. rloginconsulting.com