

Design, Synthesis, and Biological Evaluation of a Modular Polymer- based Platform for Antimicrobial Photodynamic Therapy

DISSERTATION

**zur Erlangung des Grades eines Doktors
der Naturwissenschaften**

vorgelegt von

M.Sc. Ali Balasini

eingereicht bei der Naturwissenschaftlich-Technischen Fakultät

der Universität Siegen

Siegen 2025

Betreuer und erster Gutachter

Prof. Dr. Ulrich Jonas

Universität Siegen

Zweiter Gutachter

HDR Dr. Tony Le Gall

University of BREST (Frankreich)

Weitere Prüfungskommission:

Prof. Dr. Claudia Wickleder (Universität Siegen)

Prof. Dr. Thomas Lenzer (Universität Siegen)

Tag der mündlichen Prüfung

25.03.2026

*„Chemistry is the study of matter. But I prefer to see it as
the study of change.”*

Walter White

Contents

Abstract.....	1
Zusammenfassung	3
Acknowledgements	5
Abbreviations	7
Structural abbreviations.....	10
1 Introduction	15
1.1 Background: Antimicrobial Resistance and Respiratory Infections.....	15
1.2 Photo Dynamic Therapy and its Antimicrobial Applications.....	21
1.2.1 Antimicrobial Photo Dynamic Therapy.....	23
1.2.2 Photosensitisers in Antimicrobial Photo Dynamic Therapy	25
1.3 Polymer-based Drug Delivery Systems	28
2 Research Objectives	33
3 Results and Discussion	36
3.1 Selection and Synthesis of Motifs for Antimicrobial Photodynamic Therapy.....	36
3.1.1 Functionalized Motifs for Polymer Integration	37
3.1.2 Development of Monomers for Free Radical Polymerization	43
3.2 Biofilm Disruptors Motifs.....	51
3.3 Polymerization Strategies.....	54
3.3.1 Post-Polymerization Modification (PPM)	56
3.3.2 Polymers Synthesis <i>via</i> Free Radical Polymerization (FRP).....	67
3.4 Results from Physiochemical and Biological Evaluations	75
3.4.1 Electrophoresis Test	79
3.4.2 Singlet Oxygen Sensor Green Assay	83
3.4.3 Assays using Planktonic Bacterial Cells	88
3.4.4 Assays using Bacteria in Biofilms.....	100
3.4.5 Assays using Eukaryotic Cells.....	107
3.4.6 Nebulization Studies	109
4 Conclusion and Outlook	114

5 Experimental Section	117
5.1 Chemicals	117
5.2 Instruments	119
5.3 Synthesis of Active Ester Monomers	121
5.4 Synthesis of Surfactant and Biofilm Disruptor Monomers	125
5.5 Synthesis of Phenanthroline Derivatives	128
5.6 Synthesis of Ruthenium Complexes and Subsequent Monomers.....	131
5.7 Synthesis of Phenalene-1-one and Subsequent Monomer	142
5.8 Synthesis of Polymers for Strategy 1	149
5.9 Synthesis of Polymers for Strategy 2.....	164
5.10 Failed Reactions	180
6 References	183
7 Appendix.....	202

Abstract

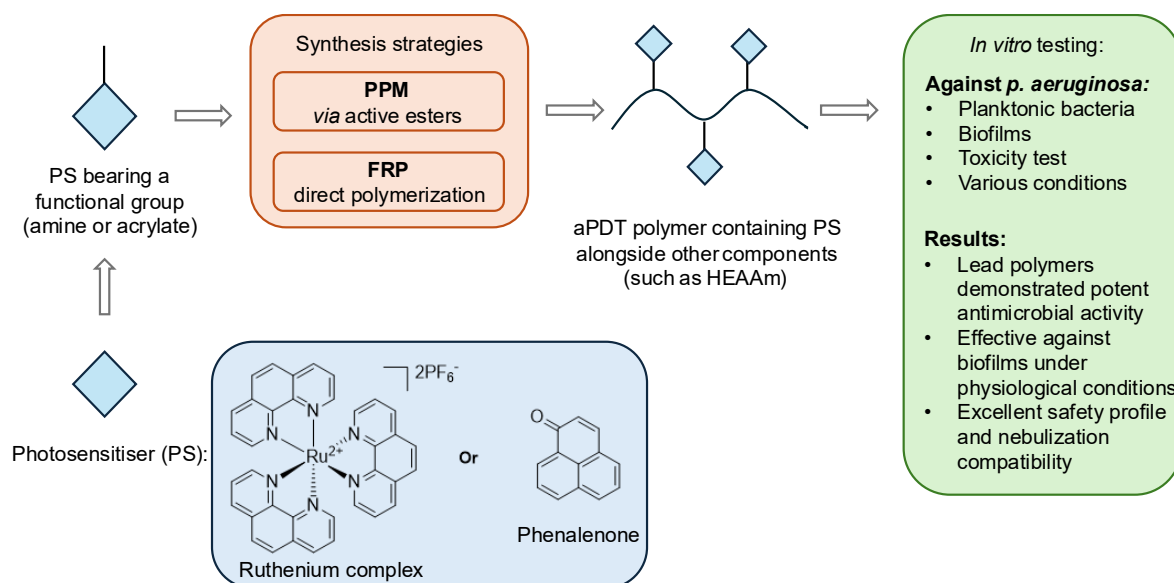
The emergence of antibiotic-resistant bacteria, particularly in respiratory infections, presents an urgent need for alternative therapeutic approaches. This thesis describes the development of polymer-based antimicrobial photodynamic therapy systems designed specifically for treating *Pseudomonas aeruginosa* respiratory infections.

Two complementary synthetic strategies were employed to create functional polymeric materials incorporating photosensitizers and other active ingredients (biofilm-disrupting agents).

The first approach involved post-polymerization modification of active ester polymers, enabling controlled incorporation of both ruthenium complexes and phenalenone derivatives as photosensitizers. The second strategy utilized direct free radical polymerization of photosensitizer-containing monomers alongside functional comonomers. Both approaches successfully yielded water-soluble polymers intended for nebulization-based delivery. Compared to free photosensitizers, these polymer-based systems offer advantages including enhanced local drug concentration through multivalent presentation and improved solubility, addressing key limitations of conventional photosensitizers.

Comprehensive biological evaluation highlighted various properties of the polymers designed with identification of structure-activity relationships. Some of these materials can effectively inactivate and/or eliminate both planktonic bacteria and biofilms upon visible light illumination, with activity maintained in physiologically relevant conditions. Notably, certain polymers also showed enhanced efficacy in high-salt environments representative for respiratory infections such as cystic fibrosis. The materials exhibited excellent biocompatibility with human bronchial epithelial cells and maintained their structural integrity and antimicrobial activity through nebulization, supporting their potential for therapeutic applications.

This work establishes a promising platform for treating respiratory infections through controlled, light-activated antimicrobial activity, offering advantages over conventional antibiotic treatments while maintaining compatibility with clinical delivery methods.



Graphical abstract: Antimicrobial photodynamic therapy polymer synthesis and biological evaluation. Two synthetic strategies, post-polymerization modification (PPM) and free radical polymerization (FRP), were used to incorporate photosensitizers into functional polymers, which were then evaluated against *P. aeruginosa*. Selected polymers showed excellent antimicrobial activity, biofilm disruption, and clinical compatibility.

Zusammenfassung

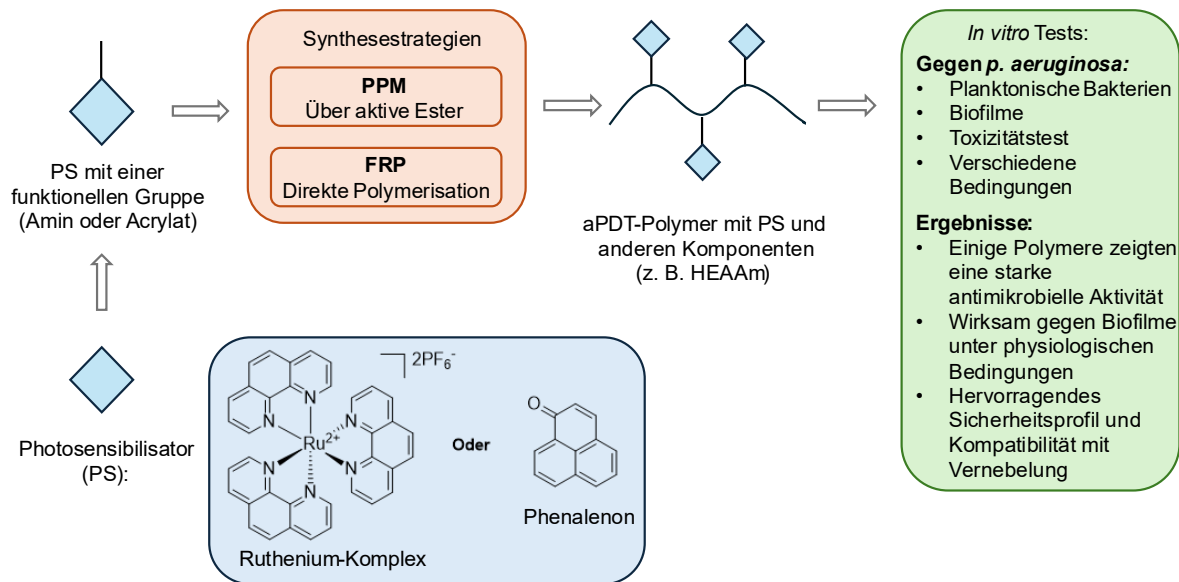
Das Auftreten antibiotikaresistenter Bakterien, insbesondere bei Atemwegsinfektionen, erfordert dringend alternative therapeutische Ansätze. Diese Dissertation beschreibt die Entwicklung polymerbasierter antimikrobieller photodynamischer Therapiesysteme, die speziell für die Behandlung von *Pseudomonas aeruginosa*-Atemwegsinfektionen konzipiert wurden.

Zwei komplementäre Synthesestrategien wurden eingesetzt, um funktionelle Polymermaterialien herzustellen, die Photosensibilisatoren und andere Wirkstoffe (Biofilm-Disruptoren Substanzen) enthalten.

Der erste Ansatz umfasste die Post-Polymerisationsmodifikation von Aktivester-Polymeren, die eine kontrollierte Einbindung sowohl von Rutheniumkomplexen als auch von Phenalenonderivaten als Photosensibilisatoren ermöglichte. Die zweite Strategie nutzte die direkte freie radikalische Polymerisation von photosensibilisatorhaltigen Monomeren zusammen mit funktionellen Comonomeren. Beide Ansätze führten erfolgreich zu wasserlöslichen Polymeren, die für die Verabreichung mittels Vernebelung geeignet sind. Im Vergleich zu freien Photosensibilisatoren bieten diese polymerbasierten Systeme Vorteile wie erhöhte lokale Wirkstoffkonzentration durch multivalente Präsentation und verbesserte Löslichkeit, wodurch wesentliche Einschränkungen konventioneller Photosensibilisatoren überwunden werden.

Eine umfassende biologische Bewertung zeigte verschiedene Eigenschaften der entwickelten Polymere und identifizierte Struktur-Wirkungs-Beziehungen. Einige dieser Materialien können sowohl planktonische Bakterien als auch Biofilme bei Lichtaktivierung effektiv inaktivieren und/oder eliminieren, wobei die Aktivität unter physiologisch relevanten Bedingungen erhalten bleibt. Bemerkenswert ist, dass bestimmte Polymere auch eine erhöhte Wirksamkeit in Hochsalzumgebungen zeigten, die typisch für Atemwegsinfektionen wie bei Mukoviszidose sind. Die Materialien wiesen eine ausgezeichnete Biokompatibilität mit menschlichen Bronchialepithelzellen auf und behielten ihre strukturelle Integrität und antimikrobielle Aktivität während der Vernebelung bei, was ihr Potenzial für therapeutische Anwendungen unterstützt.

Diese Arbeit etabliert eine vielversprechende Plattform zur Behandlung von Atemwegsinfektionen durch kontrollierte, lichtaktivierte antimikrobielle Aktivität, die Vorteile gegenüber herkömmlichen Antibiotikabehandlungen bietet und gleichzeitig mit klinischen Verabreichungsmethoden kompatibel ist.



Grafische Zusammenfassung: Synthese und biologische Bewertung antimikrobieller photodynamischer Therapie-Polymere. Zwei Synthesestrategien, Postpolymerisationsmodifikation (PPM) und Freie radikalische Polymerisation (FRP), wurden verwendet, um Photosensibilisatoren in funktionelle Polymere zu integrieren, die anschließend gegen *P. aeruginosa* getestet wurden. Ausgewählte Polymere zeigten ausgezeichnete antimikrobielle Aktivität, Biofilm-Disruption und klinische Kompatibilität.

Acknowledgements

First and foremost, I would like to express my deepest gratitude to my supervisor and mentor, Prof. Dr. Ulrich Jonas, for giving me the opportunity to work in his research group. I felt welcomed from the very first day, and his insightful discussions and guidance inspired me to learn and grow in ways I never imagined. Beyond his scientific mentorship, his approachable and down-to-earth personality made this journey truly enjoyable. I will be forever grateful for his support and encouragement.

I extend my sincere thanks to Dr. Tony Le Gall for performing all the biological evaluations presented in this thesis. He invested tremendous effort and provided invaluable guidance throughout this work. I feel fortunate to have him as a reviewer for my PhD examination. Without your contributions, Tony, this thesis would not have been possible.

My deepest thanks go to Dr. Franck Thetiot, whom I also consider a mentor. He supported me from the very beginning, offering practical solutions to countless challenges and helping me navigate difficult moments. His suggestions were instrumental in bringing this work to completion. I am also grateful for his warm hospitality during our visits to Brest – thank you, big brother (as we fondly called you)!

I am very grateful to Prof. Gilles Lemerrier and Dr. Jeremy Godard for their invaluable assistance with the synthesis and characterization of ruthenium complexes, and for generously providing key compounds used in this work.

I would like to thank Prof. Dr. Thomas Lenzer and Prof. Dr. Claudia Wickleder for serving as examiners on my thesis committee. Your time and expertise are greatly appreciated.

My sincere thanks go to Petra Franck, who was the first person to welcome me into the group. Her patience, support, and guidance in teaching me laboratory techniques were invaluable, and I am deeply grateful for her kindness throughout my time here.

I am profoundly thankful to my colleague and friend Aliia Ibragimova. She provided tremendous support during the writing phase, helping me find solutions to many challenges I faced. Your encouragement pushed me to complete this thesis even while working full-time – I couldn't have done it without you!

I would like to thank Dr. Fiona Wiesner for proofreading this thesis and helping me organize my documents, and Silvia Freese for her assistance with lab courses and administrative matters.

My thanks go to Jannik Fryn for his excellent practical work during his lab course and Master's thesis, which resulted in many valuable compounds used in this research. I also thank Tristan Zemke for his contributions to the biofilm disruptor studies, and Karla Heckhäuser for synthesizing active ester polymers.

My deepest thanks also go to Dr. Thomas Paululat for measuring countless NMRs for the whole work.

I would also like to thank Muhammed Atif for his support during my early days in the group, and former colleagues Dr. Niklas Jung, Dr. Max Meier, and Dr. Thorben Jaik for creating such a pleasant and collaborative working environment.

Beyond academia, I am grateful to Sandra vom Drop, Frau Sonja Karaman, and Frau Cosima Klocke for their support during my early years in Germany. I also extend my thanks to my colleagues at Weiss Chemie + Technik for their understanding and encouragement during the final stages of this work.

Finally, I would like to thank my family for their unwavering love, support, and belief in me throughout this journey. To my friends and everyone who has supported and encouraged me along the way – thank you from the bottom of my heart.

Abbreviations

Abbreviations	Full name
AA	acrylic acid
AIBN	azobisisobutyronitrile
AK	adenylate kinase
AMR	antimicrobial resistance
aPDT	antimicrobial photodynamic therapy
CF	cystic fibrosis
CRP	controlled radical polymerization
CTA	Chain transfer agent
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EC	<i>escherichia coli</i>
EPS	extracellular polymeric substances
ET	electron transfer
FRP	free radical polymerization
GPC	gel permeation chromatography
HEAAm	<i>N</i> -hydroxyethyl acrylamide
IC	internal conversion
ISC	intersystem crossing
LED	light-emitting diode
LD50	lethal dose, 50%
MIC	minimal inhibitory concentration
MLCT	metal-to-ligand charge transfer
NHS	<i>N</i> -hydroxysuccinimide
NMR	nuclear magnetic resonance
PA	<i>pseudomonas aeruginosa</i>
PDT	photodynamic therapy
PEI	polyethylenimine
PFP	pentafluorophenyl
PFPA	pentafluorophenyl acrylate
PFP4VBS	pentafluorophenyl 4-vinylbenzene sulfonate

PPM	post-polymerization modification
PS	photosensitizer
RAFT	reversible addition-fragmentation chain transfer
RFU	relative fluorescence unit
ROS	reactive oxygen species
SA	staphylococcus aureus
SOSG	singlet oxygen sensor green
SOSG-EP	singlet oxygen sensor green endoperoxide
Surf-EH3A	Ecosurf EH3 (surfactant with ethoxylated hydrocarbon chain)
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TTC	triphenyl tetrazolium chloride
UV-Vis	ultraviolet-visible spectroscopy
VAP	ventilator-associated pneumonia
WHO	world health organization

Physical and Chemical Symbols

Symbol	Definition
$^1\text{O}_2$	singlet oxygen
$^3\text{O}_2$	ground state (triplet) molecular oxygen
^1PS	singlet excited state of photosensitizer
^3PS	triplet excited state of photosensitizer
S_0	ground singlet state
S_1, S_2	excited singlet states
T^1	triplet state
λ	wavelength
$\phi\Delta$	quantum yield of singlet oxygen

Note on Figures, Procedures, and Nomenclature

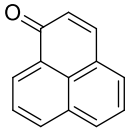
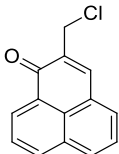
Figures without specific citations were created by the author. Figures adapted from other sources are properly cited in their respective captions. Similarly, synthetic procedures without references were developed during the course of this thesis work.

For clarity and readability, some compound and polymer names have been simplified rather than using full IUPAC nomenclature, particularly for complex structures. These simplifications maintain chemical accuracy while enhancing comprehension for readers across different disciplines.

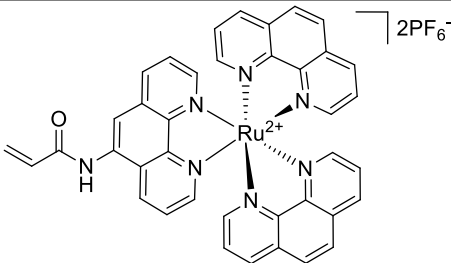
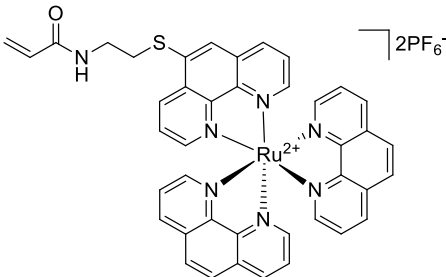
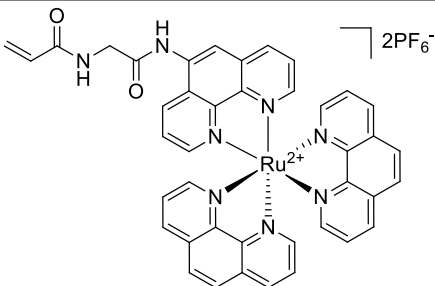
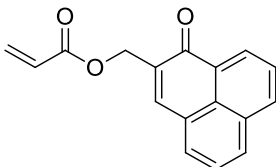
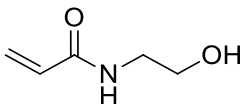
Structural abbreviations

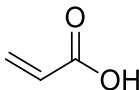
Ligands, ruthenium complexes and phenalenone derivatives

Number / Abbreviation	Full name	Structure
1	<i>N</i> -(1,10-phenanthrolin-5-yl)ethane-1,2-diamine	
2	<i>tert</i> -butyl (2-((1,10-phenanthrolin-5-yl)amino)-2-oxoethyl)carbamate	
3	2-((1,10-phenanthrolin-5-yl)thio)ethanamine	
4	1,10-phenanthrolin-monohydrat	
5 or Ru(phen)₂Cl₂	ruthenium phenanthroline) ₂ Cl ₂	(1,10-
6 or Ru(NH₂)	[ruthenium (1,10-phenanthroline) ₂ (<i>N</i> -1-(1,10-phenanthrolin-5-yl)ethane-1,2-diamine)] (PF ₆) ₂	
7 or Ru(Boc)	[ruthenium (1,10-phenanthroline) ₂ (<i>tert</i> -butyl (2-((1,10-phenanthrolin-5-yl)amino)-2-oxoethyl)carbamate)] (PF ₆) ₂	
8 or Ru(S)	[ruthenium (1,10-phenanthroline) ₂ (2-((1,10-phenanthrolin-5-yl)thio)ethanamine)] (PF ₆) ₂	
9	(1-oxo-1h-phenalen-2-yl)methanaminium chloride	

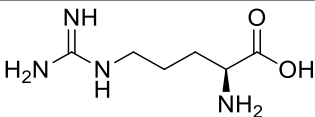
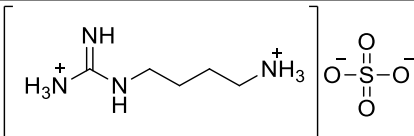
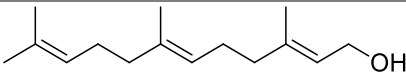
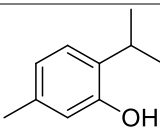
14	1H-phenalen-1-one	
15	2-(chloromethyl)-1H-phenalen-1-one	

Monomers

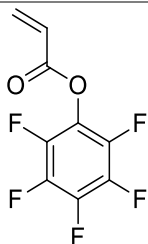
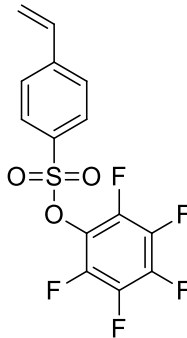
Abbreviation	Full name	Structure
11 or Ru(AA)	[ruthenium (1,10-phenanthroline) ₂ (N-(1,10-phenanthrolin-5-yl)acrylamide)] (PF ₆) ₂	
12 or Ru(S)	[ruthenium (1,10-phenanthroline) ₂ (N-(2-((1,10-phenanthrolin-5-yl)thio)ethyl)acrylamide)] (PF ₆) ₂	
13 or Ru(Boc)	[ruthenium (1,10-phenanthroline) ₂ (N-(2-((1,10-phenanthrolin-5-yl)amino)-2-oxoethyl)acrylamide)] (PF ₆) ₂	
16 or (PN)	(1-oxo-1H-phenalen-2-yl) methyl acrylate	
HEAAm	N-hydroxyethyl acrylamide	

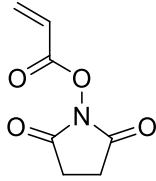
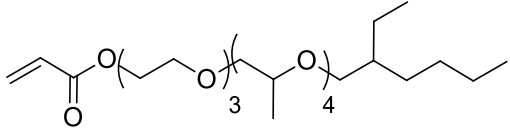
AA	acrylic acid	
-----------	--------------	--

Biofilm disruptors

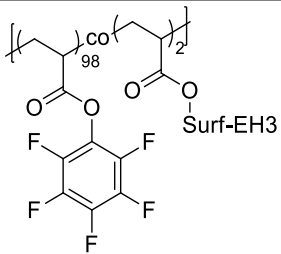
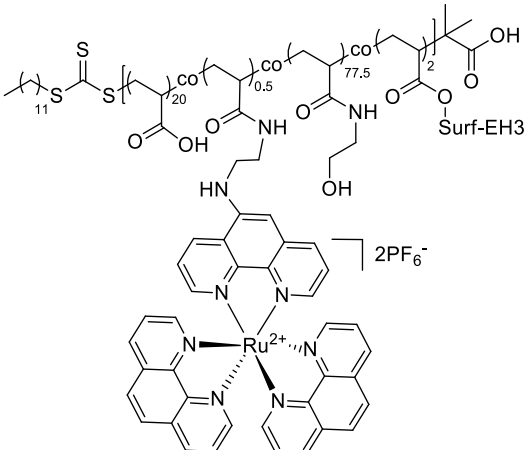
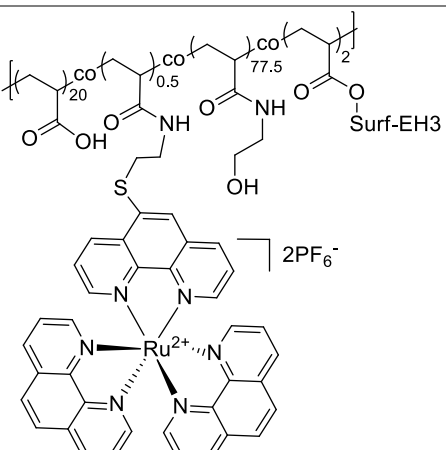
Abbreviation	Full name	Structure
17	L-arginine	
18	agmatine sulphate	
19	farnesol	
20	thymol	

Active esters and SurfEH3A

Abbreviation	Full name	Structure
21 or PFPA	pentafluorophenyl acrylate	
22 PFP4VBS	or pentafluorophenyl 4-vinylbenzene sulfonate	

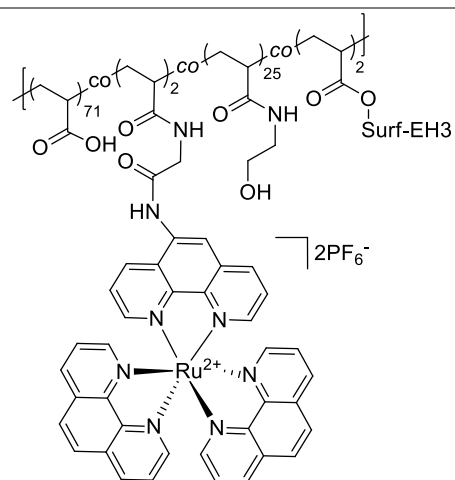
23 or NHSA	<i>N</i> -hydroxysuccinimide acrylate	
24 SurfEH3A	or ecosurf EH-3 acrylate	

Relevant polymers

Abbreviation	Full name	Structure
25	polyPFPA ₉₈ - <i>co</i> -SurfEH3A ₂	
26	poly[(AA ₂₀ - <i>co</i> -Ru(NH ₂) _{2.7} - <i>co</i> -HEAAm _{75.3} - <i>co</i> -SurfEH3A ₂)-CTA]	
27	poly(HEAAm _{77.5} - <i>co</i> -Ru(S) _{0.5} - <i>co</i> -AA ₂₀ - <i>co</i> -SurfEH3A ₂)	

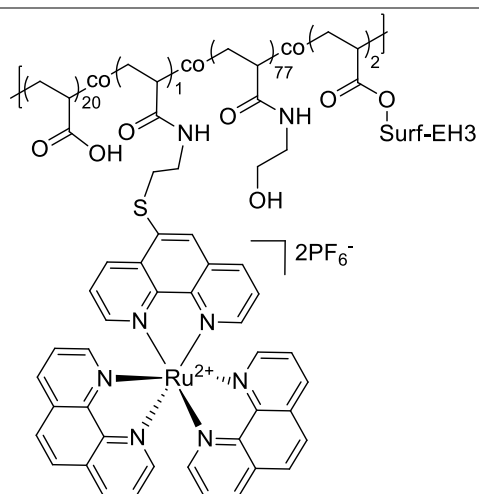
28

poly(AA₇₁-co- Ru(Boc)₂ -co-
HEAAm₂₅-co-Surf-EH3A₂)



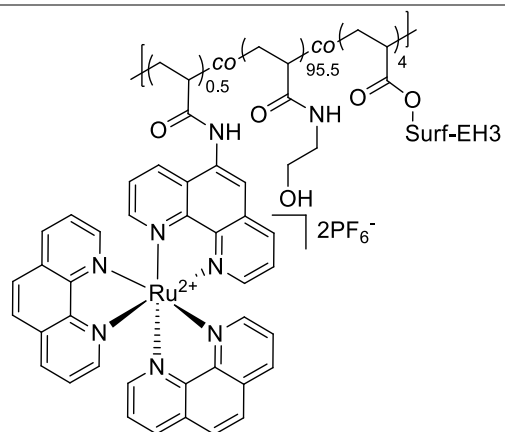
33

poly(AA₂₀-co- Ru(S)₁ -co-
HEAAm_{77.5}-co-Surf-EH3A₂)



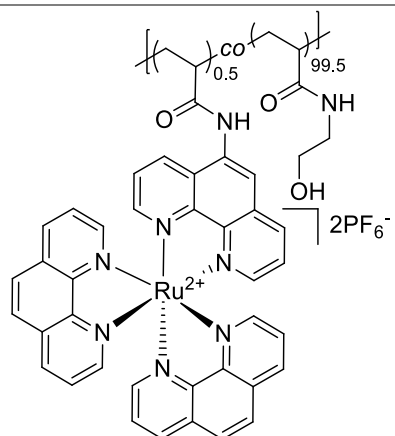
36

poly(Ru(AA)_{0.5} -co-HEAAm_{97.5}-co-
Surf-EH3A₂)



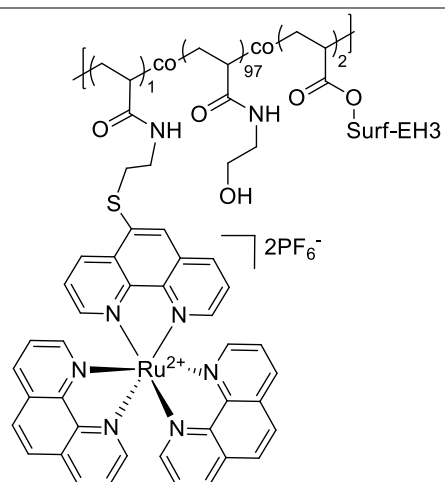
36a

poly(Ru(AA)_{0.5}-co-HEAAm_{99.5})



37

poly(Ru(S)₁-co-HEAAm_{97.5}-co-Surf-EH3A₂)



1 Introduction

1.1 Background: Antimicrobial Resistance and Respiratory Infections

The global impact of antibiotic resistance

The emergence and spread of antimicrobial resistance (AMR) represent one of the most urgent challenges in modern medicine.[1,2] The World Health Organization (WHO) has identified AMR as a critical global health threat, with conservative estimates projecting 10 million annual deaths from drug-resistant infections by 2050.[2,3]

Beyond the human cost, AMR poses substantial economic burden, with treatment costs for resistant infections expected to exceed \$100 billion annually in the United States alone.[4]

The impact of AMR extends beyond individual patient outcomes to affect entire healthcare systems and society at large.[5] The rising prevalence of resistant infections leads to prolonged hospital stays, increased mortality rates, and substantial economic burden on healthcare systems.[6,7]

Furthermore, the challenge is compounded by the limited development of new antibiotics, with pharmaceutical companies increasingly withdrawing from antibiotic research due to economic constraints.[8] This creates a critical gap between the emergence of resistant bacteria and our ability to combat them with new therapeutic options.

Respiratory Infections: A Critical Healthcare Challenge

Respiratory infections represent a particularly critical aspect of the AMR crisis, with resistant pathogens becoming increasingly prevalent in healthcare settings.[3,9] This situation is especially concerning in intensive care units, where ventilator-associated pneumonia caused by resistant organisms has become a leading cause of mortality.[6,10] The restricted penetration of conventional antibiotics into lung tissue, combined with the increasing prevalence of resistant pathogens, has created a critical need for alternative therapeutic approaches.[11]

The treatment of these infections is further complicated by the complex anatomy and multiple physiological barriers of the respiratory tract.[12,13] Understanding these barriers is crucial for developing effective therapeutic strategies.

The respiratory tract presents several key barriers to effective drug delivery as shown in Figure 1. The mucociliary clearance system actively removes foreign particles through coordinated ciliary movement.[14] Bacterial biofilms form protective communities that resist conventional treatments.[15,16] The presence of pulmonary surfactant and alveolar macrophages creates additional challenges for therapeutic delivery.[17]

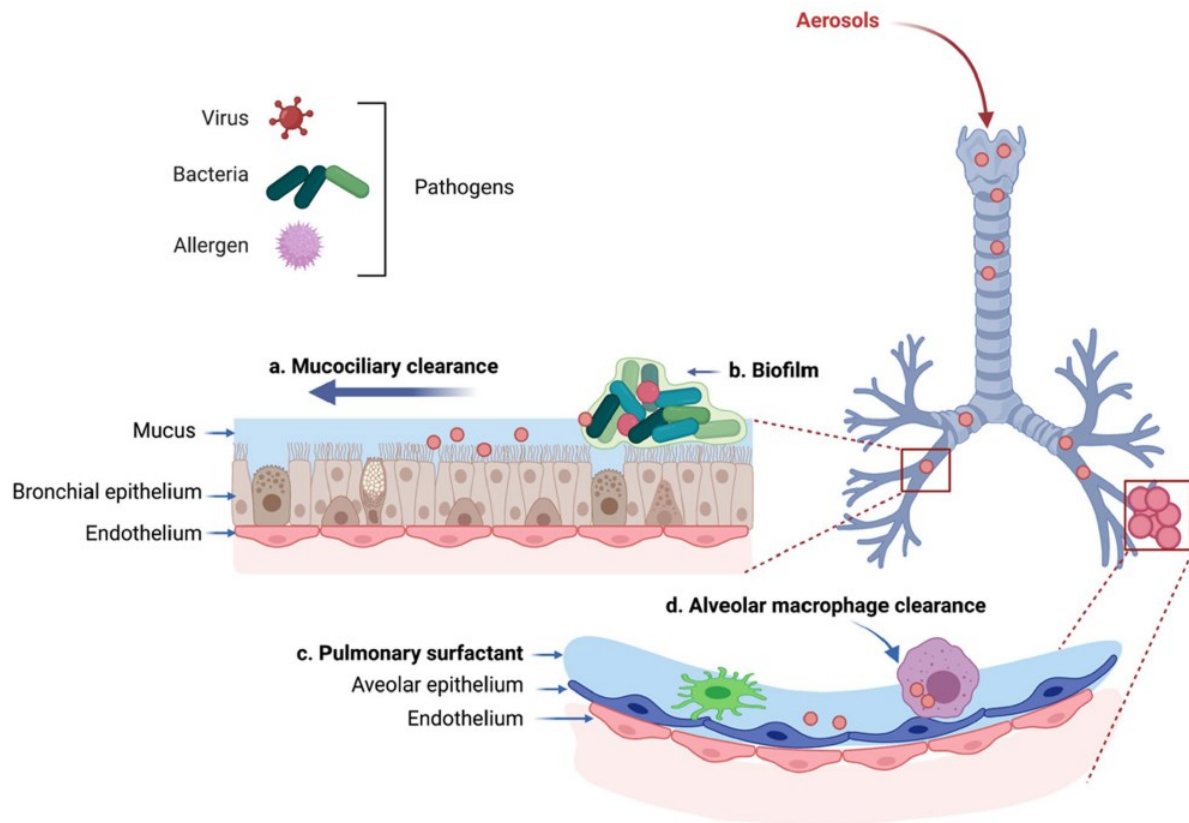


Figure 1: Key biological barriers affecting drug delivery in the respiratory tract. (a) Mucociliary clearance actively removes foreign particles, (b) bacterial biofilms form protective communities resistant to treatment, (c) pulmonary surfactant creates an additional barrier layer, and (d) alveolar macrophage clearance removes foreign materials. Adapted from He et al.[18]

These physiological barriers become particularly challenging in patients with underlying respiratory conditions. In cystic fibrosis (CF) patients, altered mucus composition creates an exceptionally difficult environment for drug delivery.[17,19] The dehydrated, highly viscous secretions not only impair natural clearance mechanisms but also create ideal conditions for bacterial colonization.[19,20] The altered airway surface liquid composition further complicates treatment by affecting drug distribution and activity.[20,21] Additionally, the thickened mucus in CF airways can create oxygen-depleted zones that favour bacterial growth and reduce the effectiveness of certain antibiotics.[19,22]

Immunocompromised patients face additional challenges in fighting respiratory infections. Their compromised natural defense mechanisms often necessitate more aggressive therapeutic approaches.[23] These patients are particularly susceptible to opportunistic pathogens that are frequently resistant to conventional antibiotics.[24] The extended duration of treatment often required in these cases, increases the risk

of developing resistant infections, creating a cycle that further complicates therapeutic management.[11,25]

Current treatment approaches, primarily relying on systemic antibiotics, face significant limitations in addressing these challenges. Poor penetration through thick mucus layers and biofilms often results in subtherapeutic drug concentrations at infection sites.[15,16,26] The rapid clearance of drugs from the airways necessitates frequent dosing or high drug concentrations to maintain therapeutic effects.[27,28] This often leads to significant systemic exposure and potential side effects, while still failing to achieve adequate drug levels at the site of infection.[27,29] Furthermore, the variable distribution of antibiotics in lung tissue can create concentration gradients that promote the development of resistance.[30,31]

These limitations highlight the urgent need for innovative delivery systems that can overcome these physiological barriers while maintaining therapeutic efficacy. Polymer-based delivery systems, particularly those designed for local activation, offer promising solutions by enabling targeted drug delivery and controlled release at infection sites.[32] The ability to design polymers with specific properties that enhance mucus penetration, resist rapid clearance, and maintain activity in the complex lung environment provides new opportunities for improving therapeutic outcomes.[32,33]

Pseudomonas aeruginosa in Respiratory Infections

P. aeruginosa, a Gram-negative bacterium, represents a critical threat in respiratory infections, particularly in healthcare settings, due to its remarkable adaptability and intrinsic resistance mechanisms.[34,35] The World Health Organization has designated it as a "high priority pathogen," reflecting both its high mortality rates and its exceptional ability to develop resistance to multiple antibiotic classes.[34,36] This pathogen is particularly problematic in respiratory infections due to its ability to thrive in the complex lung environment and form resilient biofilm communities.[35]

In chronic respiratory infections, particularly in CF patients, *P. aeruginosa* colonization represents a critical turning point in disease progression.[37] While initial lung colonization often involves other bacteria like *Staphylococcus aureus*, the exceptional adaptability of *P. aeruginosa* allows it to eventually dominate the lung environment.[38] The bacterium undergoes significant phenotypic adaptations that enhance its persistence. These adaptations include the development of mucoid phenotypes characterized by overproduction of alginate, which further protects the bacteria from both immune responses and antimicrobial treatments.[39] Additionally, the bacterium modifies its metabolism and gene expression patterns, leading to increased tolerance to oxidative stress and conventional antibiotics.[39–41] These adaptations, combined with the organism's intrinsic resistance mechanisms and biofilm-forming capabilities, make *P. aeruginosa* infections particularly difficult to eradicate using conventional therapeutic approaches.[34,39,42]

P. aeruginosa possesses multiple intrinsic resistance mechanisms that make it particularly challenging to treat. These include a highly selective outer membrane that limits antibiotic penetration, multiple efflux pump systems that actively expel antimicrobial compounds, and various enzymatic degradation mechanisms including β -lactamase production.[34] The bacterium's ability to rapidly acquire additional resistance genes through horizontal gene transfer further enhances its survival capabilities.[34] These mechanisms work synergistically to provide protection against a wide range of conventional antibiotics.[34,43]

A key feature of *P. aeruginosa* infections is the organism's exceptional ability to form biofilms, as shown in Figure 2, that represent complex bacterial communities embedded within a self-produced extracellular matrix.[44] These biofilms provide

multiple advantages for bacterial survival, including protection from host immune responses, reduced antibiotic penetration, mechanical stabilization and improved adherence of the bacterial community to the substrate tissue, and enhanced bacterial communication through quorum sensing systems.[43,44] The biofilm matrix acts as a diffusion barrier, significantly reducing the effectiveness of conventional antibiotics and necessitating novel therapeutic approaches.[16,44,45]

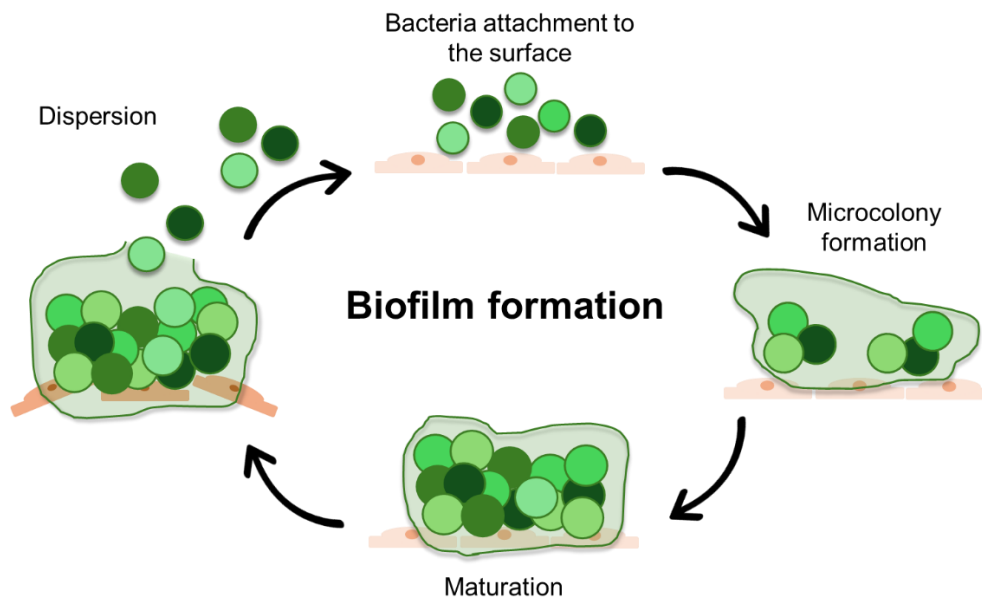


Figure 2: Stages of bacterial biofilm formation.

These characteristics of *P. aeruginosa*, particularly its ability to form resilient biofilms and develop resistance, have driven the search for alternative therapeutic strategies. Various approaches have been developed to address these challenges, including novel drug delivery systems, anti-virulence approaches, and bacteriophage therapy.[46,47] However, many of these approaches face limitations in treating respiratory infections, such as rapid clearance from the airways and insufficient distribution in infected tissue, limiting their therapeutic efficacy.[26,48,49]

Among these strategies, antimicrobial photodynamic therapy (aPDT) has emerged as a particularly promising approach due to its unique mechanism of action and potential for targeted activation.[50,51] This light-triggered therapeutic strategy offers several distinct advantages in addressing bacterial resistance, as will be detailed in subsequent sections.

1.2 Photo Dynamic Therapy and its Antimicrobial Applications

Having identified antimicrobial photodynamic therapy (aPDT) as a promising approach for treating respiratory infections, understanding its fundamental mechanisms and requirements is crucial for developing effective therapeutic strategies.

aPDT builds on the fundamental principles of photodynamic therapy (PDT). The concept of PDT emerged at the beginning of the 20th century through the pioneering observations of Oscar Raab, who discovered that certain dyes could induce cell death in the presence of light.[52,53] While this discovery initially led to developments in cancer treatment, its application in antimicrobial therapy has gained significant attention as a promising approach to combat bacterial infections.[51,54,55]

The fundamental mechanism of PDT involves three essential components: a photosensitizer, light of appropriate wavelength, and molecular oxygen. Upon absorption of light, the photosensitizer transitions from its ground state (S_0) to excited singlet states (S_1 , S_2). This excited molecule can then undergo several deactivation pathways, with intersystem crossing (ISC) to the triplet state (T_1) being crucial for photodynamic activity, as shown in Figure 3.[51,54,55] The long-lived triplet state can participate in two types of photochemical reactions:

Type I reactions involve direct electron transfer between the excited photosensitizer and biological substrates, generating reactive radical species. Type II reactions occur through energy transfer to ground state oxygen, producing singlet oxygen, a highly reactive species capable of causing oxidative damage to cellular components.[50,51,53,55]

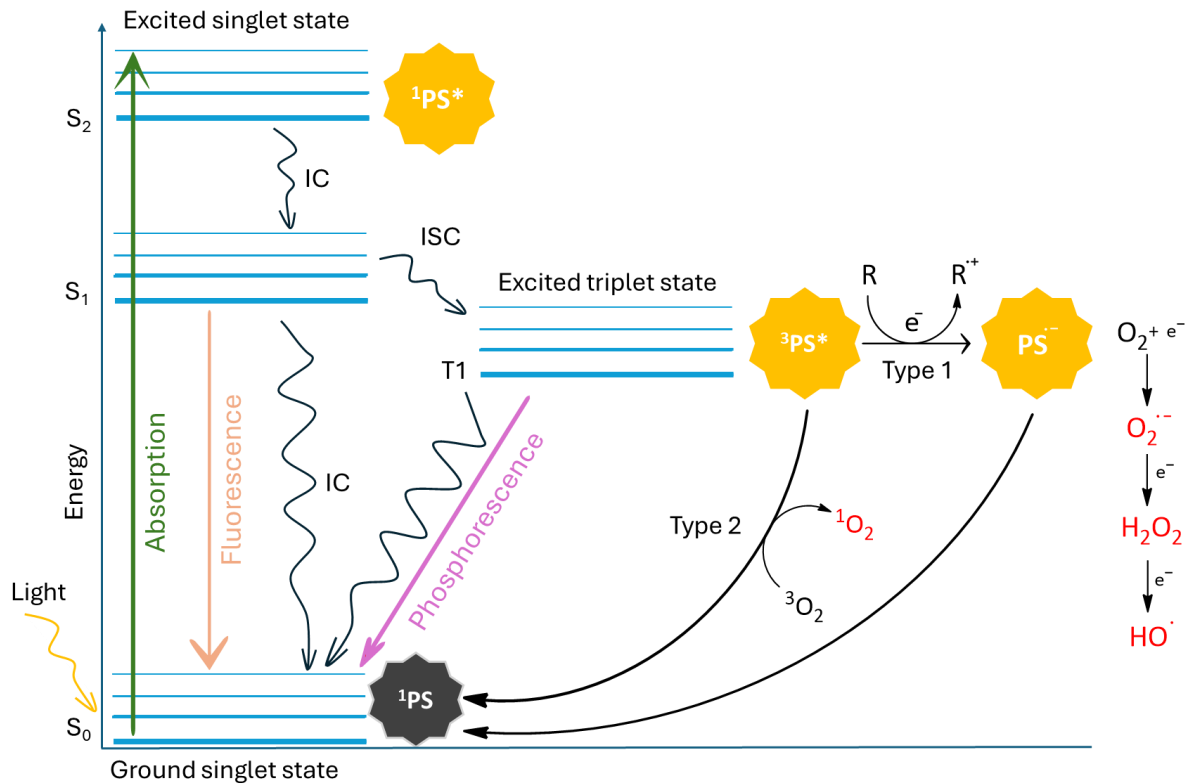


Figure 3: Jablonski diagram showing the photophysical processes and photochemical pathways in photodynamic therapy, highlighting the generation of reactive oxygen species essential for antimicrobial activity. IC: Internal Conversion; ISC: Intersystem Crossing. Created by the author, adapted from Youf et al.[51]

For respiratory applications, several factors critically influence PDT efficacy. Light delivery must balance tissue penetration depth with effective photosensitizer activation - longer wavelengths offer better tissue penetration but typically provide less energy for photosensitizer excitation.[55] The local oxygen concentration in infected tissue also impacts treatment efficacy, particularly in biofilm environments where oxygen gradients exist.[53,56]

Modern light delivery systems for PDT have evolved to address the specific challenges of respiratory infections. Fiber optic systems enable precise light delivery to infection sites through bronchoscopic approaches.[57] The development of miniaturized, flexible light delivery systems has made it possible to target specific infection sites while minimizing exposure of healthy tissue.[58] Additionally, advances in LED technology have enabled the development of more compact and efficient light sources suitable for clinical applications.[58]

1.2.1 Antimicrobial Photo Dynamic Therapy

While PDT was initially developed and established for cancer treatment, its application in antimicrobial therapy has emerged as a promising approach for combating bacterial infections, particularly those caused by resistant organisms like *P. aeruginosa*. [51,54] The adaptation of PDT principles to antimicrobial applications brings unique advantages that directly address many limitations of conventional antibiotic treatments.

The effectiveness of aPDT stems from its multifaceted mechanism of action. Unlike conventional antibiotics that typically target specific cellular processes, aPDT can simultaneously damage multiple bacterial targets through oxidative stress. [50] This broad-spectrum activity makes it particularly effective against antibiotic-resistant bacteria, as the fundamental mechanisms of antibiotic resistance provide no protection against oxidative damage. [50,59] The generated reactive oxygen species can cause:

- Disruption of bacterial cell membranes. [60]
- Oxidation of proteins and enzymes. [60,61]
- Damage to nucleic acids. [60,61]
- Interference with bacterial metabolism. [60]

A key advantage of aPDT lies in its light-triggered activation mechanism, which enables unprecedented spatial and temporal control over the therapeutic effect. [62–64] This control is particularly valuable for respiratory infections, where aPDT offers several specific advantages:

- Precise spatio-temporal control through localized light delivery. [62–64]
- Rapid antimicrobial action upon light activation. [65]
- Effectiveness against both planktonic bacteria and biofilms. [50,66]
- Minimal impact on surrounding host tissue when properly delivered. [63]

The effectiveness against bacterial biofilms relies on both efficient photosensitizer delivery and sufficient light penetration through the biofilm matrix. [32,67] These challenges have driven the development of advanced delivery systems, particularly polymer-based carriers that can enhance photosensitizer distribution within biofilms. [32] The integration of photosensitizers into polymer systems significantly enhances therapeutic potential through multiple mechanisms. These polymer-based

delivery platforms improve photosensitizer solubility and stability in biological environments while enabling controlled release and distribution at infection sites.[67] Furthermore, they enhance interaction with bacterial cells and biofilms and provide crucial protection during nebulization and delivery processes.[68–70]

The translation of aPDT to respiratory infections requires careful optimization of delivery methods. The ability to deliver photosensitizers through nebulization enables direct access to infection sites.[71,72] Combined with the light delivery systems discussed above, polymer-based photosensitizer delivery systems have been designed to maintain stability during aerosolization while preserving their photodynamic activity.[68,69,73]

1.2.2 Photosensitisers in Antimicrobial Photo Dynamic Therapy

Photosensitizers for aPDT can be broadly categorized into two main classes: organic compounds and metal complexes. Within these categories, specific attention has focused on compounds that combine high singlet oxygen quantum yields with the ability to be effectively incorporated into delivery systems.[74,75] The selection of appropriate photosensitizers for respiratory applications must consider not only their photophysical properties but also their compatibility with polymer-based delivery platforms.[67,74]

A wide range of photosensitizers have been developed for aPDT applications with specific antimicrobial activity, spanning from purely organic structures to metal complexes, some of these structures are shown in Figure 4.[51]

Organic photosensitizers include phenothiazinium dyes, porphyrins, and various aromatic systems, each offering distinct photophysical properties.[74,76] Metal-based photosensitizers, particularly ruthenium complexes, provide additional versatility through their modular synthetic nature and tunable properties of their ligands.[72,77]

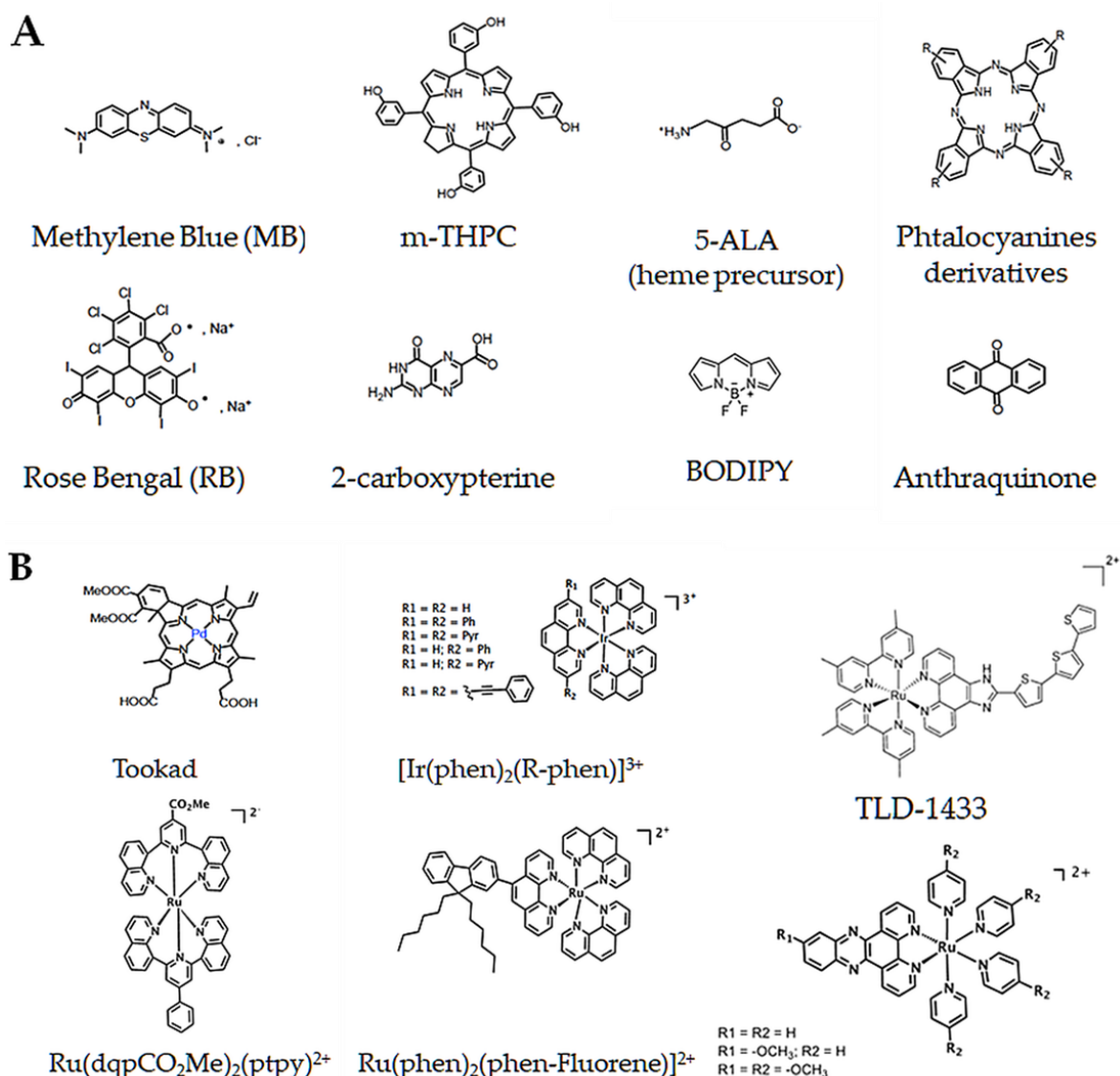


Figure 4: Representative photosensitizers with antimicrobial activity used in aPDT. (A) Organic photosensitizers including phenothiazinium dyes, porphyrins, and other aromatic systems. (B) Metal-based photosensitizers showing various ruthenium complexes with different ligand architectures. These structures illustrate the diverse molecular frameworks available for aPDT applications. Adapted from Youf et al.[51]

Among organic photosensitizers, phenalene derivatives have emerged as particularly promising candidates due to their exceptional singlet oxygen quantum yields and synthetic versatility.[78] Their relatively simple molecular structure facilitates adaptation for polymer incorporation.[78] The ability to introduce specific functional groups without compromising photophysical properties makes these compounds particularly suitable for integration into polymeric delivery systems.[78,79]

Ruthenium complexes, particularly those based on phenanthroline ligands, represent another important class of photosensitizers with distinct advantages for aPDT

applications. These metal complexes offer high photostability under prolonged irradiation and possess long-lived excited states that enhance singlet oxygen generation efficiency.[77,80] The phenanthroline ligand system is particularly valuable due to its robust coordination chemistry with ruthenium and its ability to create complexes with strong absorption in the visible region.[77,81] Their modular synthetic nature allows for strategic modification of the phenanthroline ligands to introduce specific functional groups, enabling both fine-tuning of photophysical properties and efficient polymer conjugation.[82,83] This structured synthetic chemistry provides precise control over both the photodynamic activity and the integration into polymer delivery systems.

The selection of photosensitizers for respiratory applications requires careful consideration of multiple interrelated factors. Photosensitizer stability in biological media and during nebulization is crucial for maintaining therapeutic efficacy throughout the delivery process.[71,72,84] The specific environment of the airways, particularly their high salt content and variable oxygen levels, necessitates photosensitizers that can maintain their activity under these challenging conditions.[72] Furthermore, the photosensitizer must be compatible with the synthetic procedures of polymer conjugation chemistry while retaining its ability to efficiently generate reactive oxygen species under physiological conditions.[67,85,86]

This combination of appropriate photosensitizer selection with effective delivery strategies becomes particularly crucial for treating respiratory infections, especially those involving bacterial biofilms where both penetration and activation must be optimized.

1.3 Polymer-based Drug Delivery Systems

Polymer-based delivery platforms offer diverse architectural possibilities for photosensitizer delivery. These systems can range from simple polymer-drug conjugates to more complex structures such as micelles, dendrimers, and polymersomes shown in Figure 5, each providing unique advantages for drug delivery.[51] The ability to functionalize these carrier systems with targeting ligands while incorporating photosensitizers enables the development of sophisticated delivery platforms that can enhance therapeutic efficacy.[67]

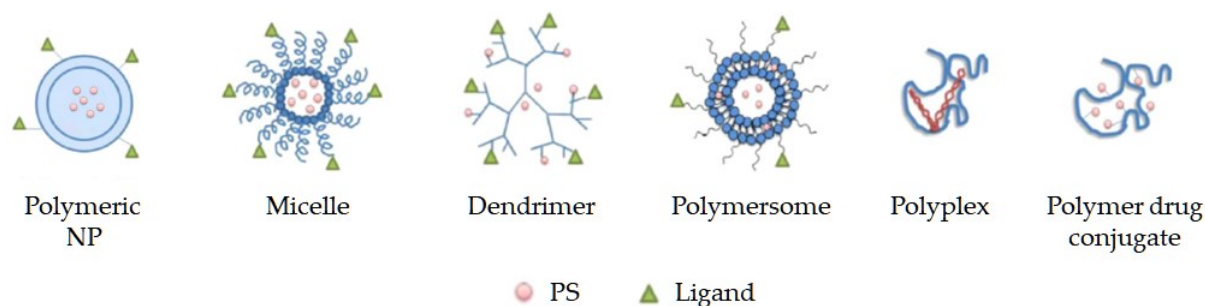


Figure 5: Different architectures of polymer-based photosensitizer delivery systems. Schematic representation of various carrier types including polymeric nanoparticles (NP), micelles, dendrimers, polymersomes, polyplexes, and polymer-drug conjugates. These architectures can be functionalized with targeting ligands and loaded with photosensitizers (PS) to create tailored delivery systems for specific applications. Adapted from Youf et al.[51]

Among these various architectures, covalent polymer-drug conjugates offer particular advantages for respiratory delivery due to their chemical stability, controlled size, tunable properties such as solubility in aqueous environments, and ability to maintain drug (photosensitizer) activity.[87] The successful development of such systems requires careful consideration of both the synthetic approach and the targeted polymer properties.

The development of polymer-based photosensitizer delivery systems can be approached through two main synthetic strategies: post-polymerization modification (PPM) of a polymeric precursor with aPDT units and direct copolymerization of photosensitizer-containing comonomers with the desired main monomers, for example by free radical polymerization (FRP). Each approach offers distinct advantages and challenges in the context of aPDT applications. Post-polymerization modification provides precise control over polymer composition and allows for the incorporation of multiple functional components. Free radical polymerization, on the other hand, offers exceptional versatility with high tolerance toward various solvents,

functional groups, and impurities, while enabling the polymerization of a wide range of vinylic monomers.[88–90]

The success of these strategies relies critically on the selection of appropriate polymerization methods. Different synthetic approaches offer distinct advantages and limitations, particularly when incorporating functional molecules such as photosensitizers and biofilm-disrupting agents.[67,87]

Polymerization methods

Free radical polymerization (FRP) represents an ideal choice for synthesizing photosensitizer-containing polymers due to several key advantages. The method offers high tolerance toward different chemical functionalities present in the monomers and photosensitizers, enabling direct incorporation of complex molecules like ruthenium complexes and biofilm-disrupting agents.[91,92] Its practical benefits include simpler experimental setup compared to controlled polymerization techniques and higher tolerance to trace impurities and moisture.[92] These characteristics make FRP particularly suitable for scaling up production of therapeutic polymers.

While FRP was the primary method used in this work, controlled radical polymerization (CRP), specifically Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization, was also employed for some polymers. RAFT represents one type of CRP, alongside other techniques like Atom Transfer Radical Polymerization (ATRP) and Nitroxide-Mediated Polymerization (NMP).[92,93] RAFT polymerization enables precise control through a chain transfer agent that mediates the polymerization *via* a reversible chain transfer process, resulting in well-defined polymers with narrow molar mass distributions.[94,95]

However, due to the inherent complexity of incorporating functional photosensitizers into polymer systems and practical considerations for potential scale-up, conventional FRP often presents a more accessible and versatile approach for synthesizing photosensitizer-containing polymers, despite the advantages in molecular control offered by CRP techniques.

The typical FRP process employs thermal initiators such as azobisisobutyronitrile (AIBN), which decomposes at 60-70°C to generate free radicals that initiate polymerization.[96] Critical experimental considerations include removal of oxygen (a

radical scavenger) through argon purging or freeze-pump-thaw cycles, and subsequent polymer purification through precipitation or dialysis to remove unreacted monomers and initiator residues.[97] [96][97]

Beyond direct polymerization techniques, post-polymerization modification (PPM) offers an alternative and versatile strategy for incorporating functional groups onto pre-formed polymer backbones.[98] Among various PPM approaches, active ester chemistry has emerged as a particularly powerful tool due to its high efficiency and versatility.[99] Active ester polymers contain activated carboxylic acid derivatives that readily undergo nucleophilic substitution with primary amines under mild conditions.[99]

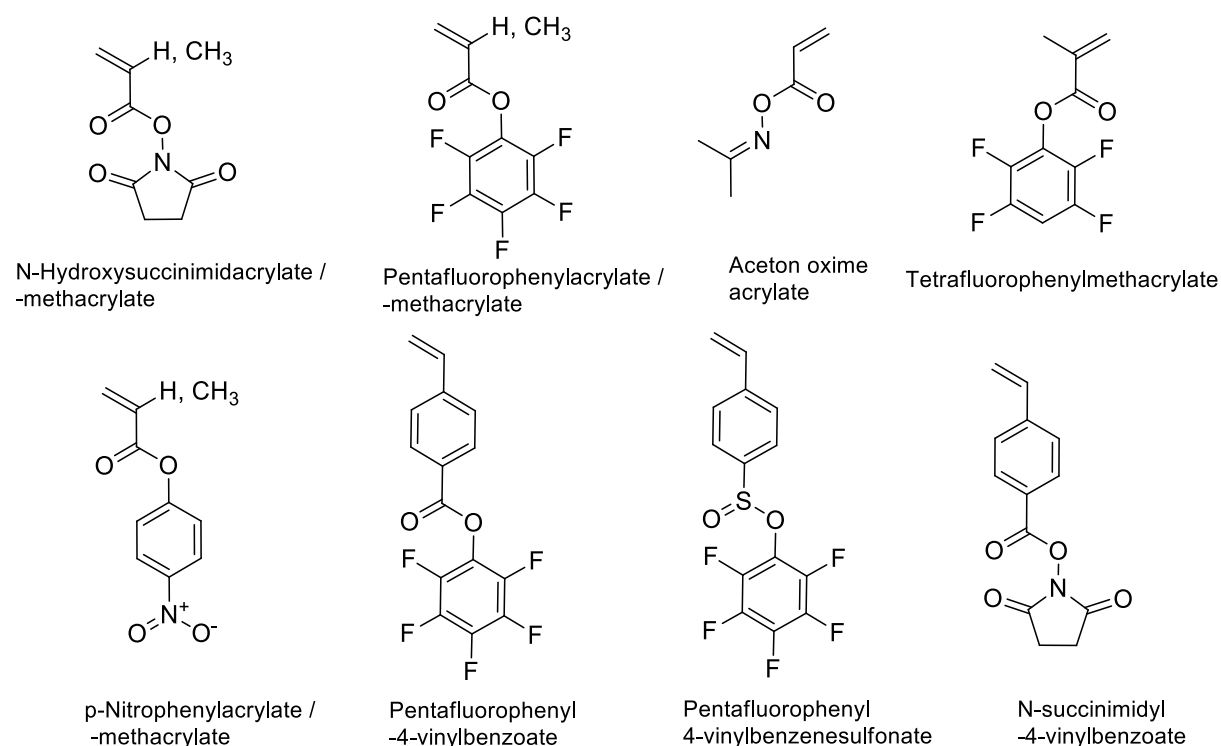


Figure 6: Common active ester structures including pentafluorophenyl esters, *N*-hydroxysuccinimide esters, and activated sulfonates.[99,100]

Among the active esters, which are shown in Figure 6, *N*-hydroxysuccinimide (NHS) esters represent the most widely used and well-established system for polymer modification.[100] Their widespread adoption stems from commercial availability, good reactivity with primary amines, and extensive documentation in bioconjugation chemistry.[100,101] However, NHS esters face certain limitations, particularly their susceptibility to hydrolysis and restricted solubility in organic solvents.[102]

These limitations have driven the development of alternative active ester systems. Pentafluorophenyl (PFP) esters have emerged as particularly valuable alternatives, offering several advantages over traditional NHS systems. PFP esters demonstrate enhanced hydrolytic stability while maintaining excellent reactivity towards primary amines.[99,103] Additionally, their broader solubility profile enables modifications in various organic solvents, expanding the range of possible modification reactions.[99,100]

The choice of active ester system significantly influences both the modification efficiency and the overall synthetic strategy. While NHS esters excel in aqueous conditions commonly used for biomolecule conjugation, PFP esters prove particularly advantageous for modifications requiring organic solvents or extended reaction times.[99,100] Understanding these characteristics is crucial for designing effective polymer modification strategies.

Additionally, the success of active ester modification depends on several key factors including the structure of the incoming nucleophile, reaction conditions, and polymer architecture. For complex molecules such as photosensitizers, considerations like steric hindrance and electronic effects become particularly important due to their molecular complexity.

Polymer design considerations

The design of polymers for photosensitizer delivery in respiratory applications requires careful consideration of multiple factors. The polymer architecture significantly influences interaction with bacterial cells and overall effectiveness of aPDT.[67,104] Key considerations include the polymer's molar mass, charge characteristics, and hydrophilic-hydrophobic balance.[105,106] Selection of appropriate polymer components is crucial for achieving desired therapeutic properties. Hydrophilic polymers like poly(*N*-hydroxyethyl acrylamide) (or polyHEAAm) have emerged as valuable building blocks due to their biocompatibility, antifouling characteristics, and suitable hydrophilicity for respiratory applications.[107,108]

The respiratory tract presents unique barriers that must be addressed through polymer design. Previous studies of polymer-based drug delivery systems for pulmonary applications have identified several critical requirements. The polymer systems must be designed with appropriate size ranges for effective airway distribution and surface

properties that enable mucus penetration.[109–111] Additionally, they must maintain stability in the presence of ions and enzymes found in respiratory secretions while remaining compatible with nebulization-based delivery methods.[69,110]

For antimicrobial photodynamic therapy applications, additional design elements become crucial. The polymer structure must maintain photosensitizer activity while providing protection during delivery.[32,47,87] The incorporation of surface-active components can help modulate polymer-bacteria interactions and enhance distribution within biofilms.[67] Additionally, the polymer structure must be designed to maintain photosensitizer accessibility to environmental oxygen, which is crucial for photodynamic efficiency.[112]

The presence of bacterial biofilms adds another layer of complexity to polymer design. The polymer architecture must facilitate penetration through the biofilm matrix while maintaining photosensitizer availability for activation.[26,67] The integration of biofilm-disrupting elements within the polymer structure offers potential for enhanced therapeutic efficacy through multiple mechanisms of action.[113] Understanding these design requirements provides crucial guidance for optimizing polymer-based delivery systems.

2 Research Objectives

As part of the Target Therapy project*, this thesis focuses on developing polymer-based antimicrobial photodynamic therapy systems specifically designed for treating *P. aeruginosa* respiratory infections. This work addresses the growing challenge of antimicrobial resistance through innovative therapeutic approaches that can overcome bacterial resistance mechanisms while enabling controlled, localized treatment, as illustrated in Figure 7, which outlines the chemical strategy and therapeutic protocol.

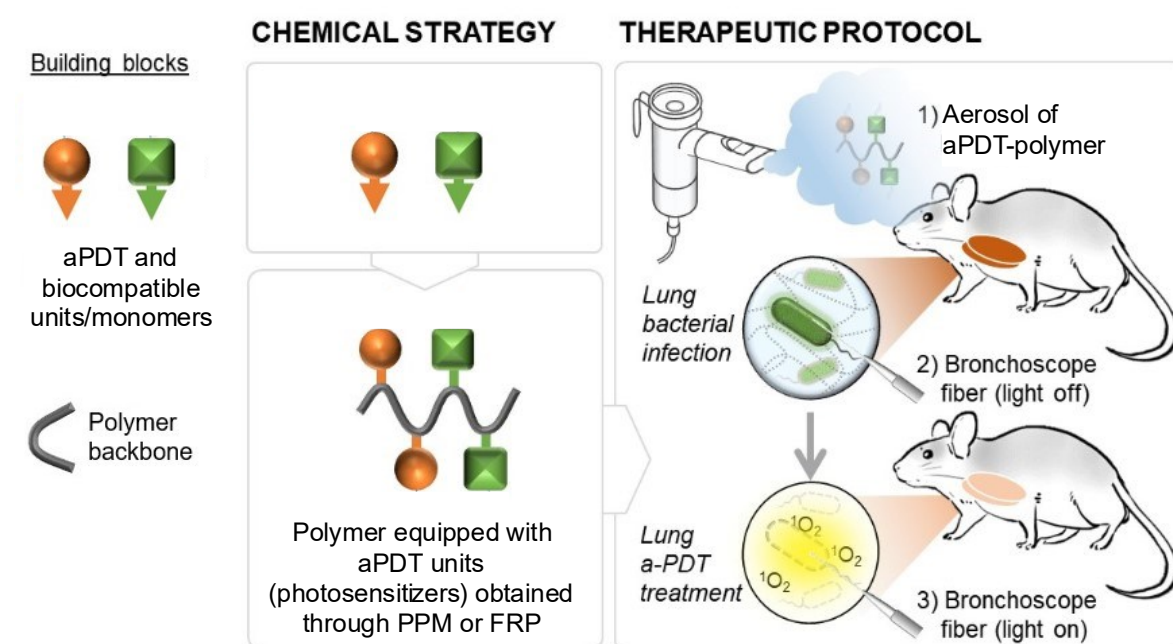


Figure 7: Overview of the polymer-based antimicrobial photodynamic therapy approach. Building blocks represent the fundamental components including photosensitizers (orange) and biocompatible units (green) that are incorporated into polymer systems. The chemical strategy illustrates the development of functional polymers equipped with both photosensitizing and biocompatible units. The therapeutic protocol demonstrates the intended application: (1) delivery of the aPDT polymer via nebulization to infected lung tissue, (2) localization of the polymer at the infection site, and (3) activation by light delivered through a bronchoscope to generate reactive oxygen species for bacterial elimination.

Based on the current challenges and opportunities identified in the field, this work focuses on four key objectives:

* The Target Therapy project is a French-German collaborative project developing antimicrobial photodynamic approaches for respiratory infections: <https://nouveau.univ-brest.fr/target-therapy-project/en>

1. Development of Advanced Photosensitizer Systems

The first objective involves the strategic design and synthesis of both ruthenium-based and organic photosensitizers suitable for polymer incorporation. This dual approach aims to leverage the distinct advantages of both metallic and organic photosensitizers while enabling their integration into polymer delivery systems.[51] Particular attention is given to maintaining high singlet oxygen generation efficiency while introducing functional groups necessary for polymer attachment.

2. Design of Multifunctional Polymer Platforms

A central aim is to develop polymer systems capable of both efficient photosensitizer delivery and biofilm disruption. This involves exploring two complementary synthetic strategies: post-polymerization modification and direct polymerization of photosensitizer-containing monomers. The incorporation of biofilm-disrupting agents alongside photosensitizers aims to enhance therapeutic efficacy through complementary mechanisms of action.

3. Optimization for Respiratory Delivery

The development of nebulizer-compatible formulations represents a crucial objective, requiring careful optimization of polymer properties to maintain stability and function during aerosolization. This includes the incorporation of surface-active components to enhance interaction with bacterial biofilms while maintaining appropriate solubility and distribution characteristics. The biological evaluation of nebulization compatibility was conducted in collaboration with Dr. Tony Le Gall at the University of Brest, France.

4. Comprehensive Biological Evaluation

The final objective encompasses thorough evaluation of these systems against *P. aeruginosa* under conditions relevant to respiratory infections. This includes assessment of both planktonic and biofilm forms of bacteria, investigation of the impact of biological matrices, and evaluation of biocompatibility with respiratory epithelial cells. This comprehensive biological evaluation was also performed through a joint collaboration with Dr. Tony Le Gall at the University of Brest, France, as this specialized work can only be conducted at their facilities.

Through these objectives, this work aims to contribute to the development of more effective treatments for resistant respiratory infections while advancing our understanding of polymer-based aPDT systems.

Having established the fundamental principles and current challenges in antimicrobial photodynamic therapy, the following chapters present our systematic approach to developing effective polymer-based aPDT systems.

The successful development of these polymer-based aPDT systems could provide a significant advance in treating resistant respiratory infections, particularly in patients where conventional antibiotics have limited efficacy.

3 Results and Discussion

This chapter is divided into two major sections: the chemical synthesis and characterization of the materials, followed by their comprehensive biological evaluation. This first section focuses on the synthetic strategies employed to develop the targeted aPDT systems. The synthetic work progressed through multiple stages, beginning with the preparation of photosensitizer units and their subsequent integration into polymer systems through both PPM and FRP approaches. Each synthetic step was carefully optimized and isolated products characterized to ensure the resulting materials possessed the desired structural and functional properties. The biological evaluation of the target materials, including their antimicrobial efficacy and biocompatibility, is presented in the subsequent section.

3.1 Selection and Synthesis of Motifs for Antimicrobial Photodynamic Therapy

The selection of appropriate aPDT units was crucial for this thesis work. While various photosensitizers could potentially serve this purpose, this study focused specifically on two distinct classes: organometallic ruthenium complexes and phenalenone as an organic photosensitizer. These compounds were chosen based on their complementary properties and potential synergistic effects in aPDT applications.

The ruthenium complexes were selected for their well-documented photophysical properties, including strong absorption in the visible region, long-lived excited states, and efficient singlet oxygen generation.

Additionally, their modular synthetic nature allowed for the introduction of specific functional groups necessary for polymer incorporation. Phenalenone, as an organic alternative, offered advantages including high quantum yields of singlet oxygen production and synthetic versatility.

Both photosensitizer types were designed to include specific functionalities that would enable their subsequent incorporation into polymer systems.

The following sections detail the synthetic methodologies employed for each photosensitizer type, their characterization, and the optimization of reaction conditions to achieve the desired functionalized products suitable for polymer conjugation.

3.1.1 Functionalized aPDT Motifs for Polymer Integration

The synthesis of aPDT motifs focused on developing photosensitizers with specific functional groups suitable for PPM. A key design requirement was the incorporation of primary amine groups, which would enable subsequent conjugation to active ester units in the polymer backbones. The synthetic strategy began with the preparation of functionalized phenanthroline ligands, which served as precursors for the ruthenium complexes.

Phenanthroline-based ruthenium complexes

The phenanthroline ligand system was specifically chosen for its robust coordination chemistry with ruthenium and its ability to create complexes with strong absorption in the visible region.[82,114–116] The modular nature of phenanthroline chemistry also allowed systematic variation of spacer length and type, enabling improvement of both synthesis and final polymer properties.

A critical synthetic challenge encountered in this work was the limited reactivity of primary amines groups directly attached to aromatic rings in PPM reactions, which is the case for the commercially available 5-amino-1,10-phenanthroline.[99,117,118] These aromatic primary amines demonstrate significantly reduced nucleophilicity compared to aliphatic primary amines, due to electron delocalization within the aromatic system that lowers the electron density on the nitrogen lone pair. This electronic deficiency prevents effective interaction with active ester groups in polymer modification. To overcome this limitation, a strategic approach was developed involving the incorporation of alkyl spacers between the aromatic core and the amine functionality. These alkyl spacers serve two crucial purposes: first, they disrupt the electronic conjugation that inhibits nucleophilic attack, and second, they introduce conformational flexibility that facilitates more efficient polymer modification reactions. By introducing these alkyl spacers into the ligands, the reactivity of primary amines is significantly enhanced, enabling successful post-polymerization modifications that were previously challenging.

The synthesis of phenanthroline ligands was accomplished through carefully optimized procedures that introduced aliphatic amine functionalities with varying alkyl spacer lengths. Three key ligands, which are shown in Figure 8, were successfully synthesized with each requiring specific optimization approaches; the synthesis of *N*-

(1,10-Phenanthroline-5-yl)ethane-1,2-diamine (**1**) was enhanced in this work through microwave-assisted conditions compared to literature procedures, reducing reaction time from overnight to 3 hours while maintaining high yields.[119]

Modification of the purification protocol for tert-butyl(2-((1,10-phenanthroline-5-yl)amino)-2-oxoethyl)carbamate (**2**) through addition of a basic washing step led to yields approaching 90%.[120] A modified purification protocol of 2-((1,10-phenanthroline-5-yl)thio)ethanamine (**3**) significantly improved product yield from 60% to over 80%.[114] The full synthetic procedures for all ligands are detailed in the experimental section (5.5).

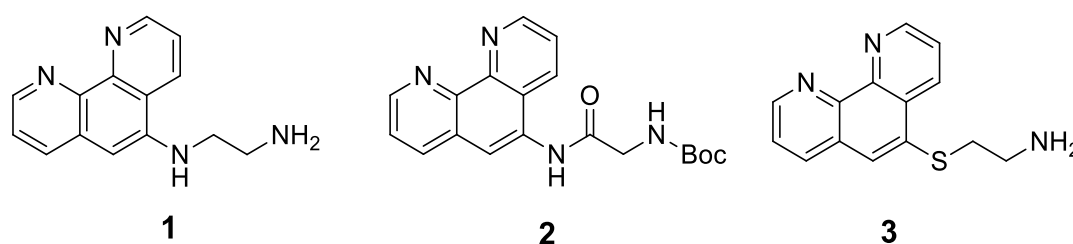


Figure 8: Ligands synthesized bearing different functional groups.

Successful synthesis of these ligands was confirmed through the ^1H NMR spectra, in which unambiguous signal assignments allowed identification of both aromatic protons of the phenanthroline core and the aliphatic protons of the spacer unit (e. g. Fig. SI 13 in Appendix).

Having successfully synthesized the phenanthroline ligands with their tailored functional groups, the next step involved their incorporation into ruthenium complexes. This required the preparation of a suitable ruthenium precursor that could coordinate with these functionalized ligands.

The synthesis of the precursor complex $\text{Ru}(\text{phen})_2\text{Cl}_2$ (**5**) was then undertaken following established literature procedure[121] with several key considerations: the reaction was conducted in DMF at 150°C with RuCl_3 and 1,10-phenanthroline (**4**) in the presence of LiCl , yielding the desired complex in 30-40% yield, as shown in Figure 9.

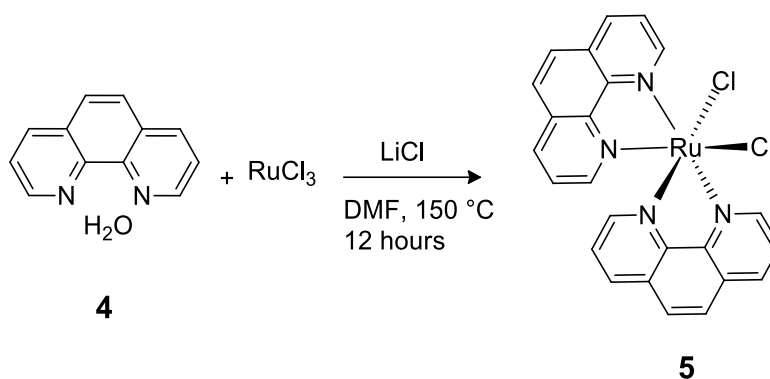


Figure 9: Synthesis of Ru(1,10-Phenanthroline)₂Cl₂ (**5**).

A significant challenge was the limited solubility of the product, necessitating careful optimization of reaction and isolation conditions. The product was effectively isolated through a precipitation protocol, followed by further purification *via* extraction. The purified precursor complex was used directly in subsequent reactions without additional modification, as confirmed by ¹H NMR analysis.

The coordination of functionalized phenanthroline ligands to the Ru(phen)₂Cl₂ precursor complex (**5**) was conducted under optimized conditions (adapted from Lemerrier et al.[115,116]), carefully developed to ensure high yields and product purity. The complexation reactions were performed in anhydrous ethanol under reflux (78°C) for 16-20 hours, with reaction progress monitored *via* thin-layer chromatography. Following coordination, counter-ion exchange was achieved through the addition of saturated NH₄PF₆ solution at ambient temperature, resulting in the precipitation of ruthenium (II) complexes as their hexafluorophosphate salts. The products were then purified through a liquid-liquid extraction system using dichloromethane and water, providing the pure complexes suitable for subsequent PPM reactions. The complexes are shown in Figure 10.

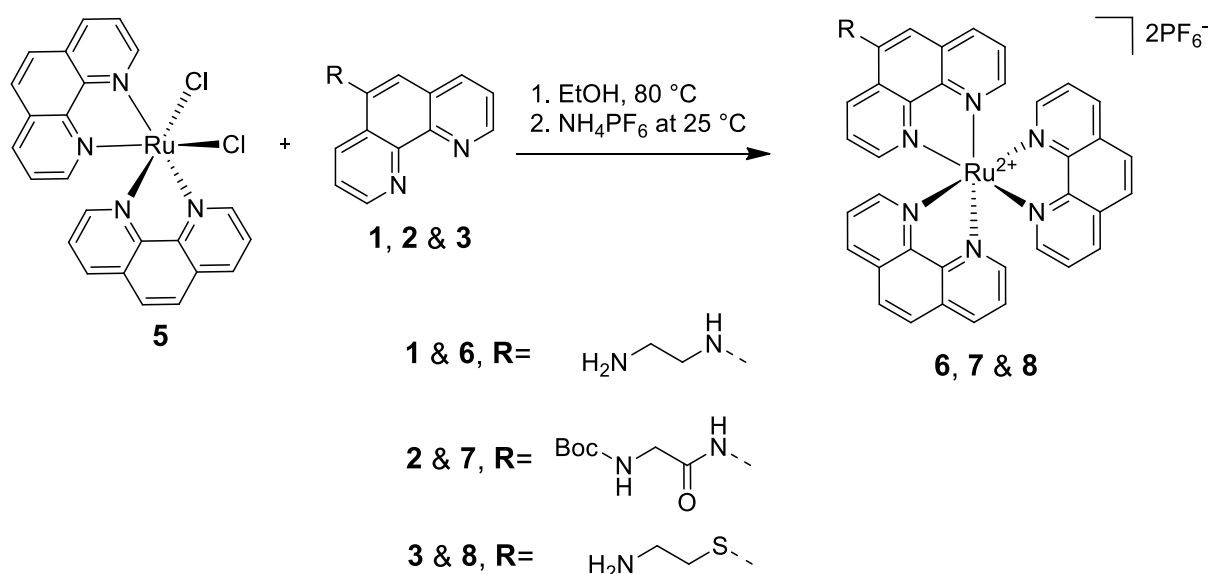


Figure 10: General synthesis route of the complexes **6**, **7** & **8**.

The synthetic outcomes varied notably among different target complexes. Compound **6** was synthesized by Prof. Gilles Lemerrier from the Paris Cité University (UPC). Ligand **1** was partially protonated by hydrobromic acid and showed diminished coordination efficiency due to protonation not only at the primary amine but also of the aromatic nitrogens in the phenanthroline ring system. This protonation hindered effective coordination with the ruthenium center, resulting in lower yields for this complex.

Compound **7** required careful selection of the deprotection strategy. Two alternative approaches were investigated for Boc group removal: trifluoroacetic acid (TFA) in dichloromethane and HCl gas bubbling through a methanolic solution. While TFA successfully removed the Boc group, the resulting TFA salts were difficult to remove during purification, compromising product purity. The HCl-mediated deprotection proved better, offering enhanced selectivity and product purity while eliminating the need for additional purification steps. This method's advantage lay in the production of only volatile by-products (CO_2 , isobutylene, and HCl), simplifying the isolation process.

In contrast, compound **8** demonstrated superior reactivity and straightforward purification, consistently achieving isolated yields of $60 \pm 5\%$ across multiple synthetic batches. This reproducibility was particularly valuable for scaling up the synthesis. The full synthetic procedures for all ligands are detailed in the experimental section (5.6).

The successful synthesis and purity of these complexes were confirmed through comprehensive characterization techniques. ^1H -NMR spectroscopy provided clear evidence of ligand coordination and complex formation, with characteristic shifts in the aromatic region corresponding to the coordinated phenanthroline ligands. UV-Visible absorption spectroscopy further confirmed successful complex formation through the observation of the absorption band at approximately 450 nm, typical for ruthenium (II) complexes, for details see Figure 38 in chapter 3.4 Results from Physiochemical and Biological Evaluations.

Phenalenone system

As an organic alternative to ruthenium complexes, phenalenone derivatives were selected for their high singlet oxygen quantum yields and simpler molecular structure.[78,79] Similar to the strategy employed with ruthenium complexes, an aliphatic primary amine functionality was incorporated into the phenalenone structure to enable efficient polymer conjugation. This design choice addressed the previously discussed reactivity limitations of aromatic-bound amines while preserving the compound's photosensitizing properties.

The phenalenone derivative, (1-oxo-1*H*-phenalen-2-yl)methanaminium (**9**) chloride, which is shown in Figure 11, was obtained from our collaboration partner Dr. Jeremy Godard at the Paris Cité University (UPC). This compound served as a complementary organic photosensitizer to the ruthenium complexes for post-polymerization modification reactions.[78]

The synthetic route to the target compound involved a multi-step procedure starting from 1*H*-phenalen-1-one. The synthesis proceeded through initial functionalization *via* alkyl halide introduction, followed by nucleophilic substitution using sodium azide.

The resulting azido intermediate was then converted to the corresponding primary amine through a Staudinger reduction using triphenylphosphine in THF/H₂O, followed by hydrolysis. The final product was isolated as its hydrochloride salt to enhance stability and facilitate handling.[78]

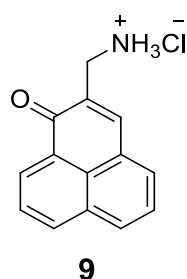


Figure 11: Phenalenone derivative **9** for PPM synthesis.

3.1.2 Development of Monomers for Free Radical Polymerization

The development of aPDT monomers suitable for direct free radical polymerization represented a crucial synthetic strategy for incorporating aPDT units into polymer systems. This approach offered an alternative to post-polymerization modification, enabling direct polymerization of photosensitizing units alongside other monomers (e. g. HEAAm). By designing monomers with polymerizable functional groups, a more streamlined synthetic route could be achieved, potentially allowing for more uniform distribution of photosensitizer units within the polymer backbone. The same photosensitizer used for strategy 1 (PPM in chapter 3.3.1) were targeted for monomer development, ruthenium complexes and phenalene derivatives, each requiring careful modification to introduce polymerizable functionality, for strategy 2 (chapter 3.3.2), while maintaining their essential photophysical properties.

Monomer synthesis with ruthenium complexes

A series of acrylamide monomers containing ruthenium complexes was successfully synthesized. The design incorporated both aromatic and aliphatic amine-functionalized complexes, each converted to their corresponding acrylamide derivatives. This synthetic effort yielded three acrylamide monomers, two of which (**11** and **12**) are shown in Figure 12. Compound **11** has the acrylamide directly attached to the phenanthroline core, whereas compound **12** incorporates a thioether spacer. The spacer in **12** reduces steric hindrance during propagation, leading to more efficient monomer incorporation in free radical polymerization.

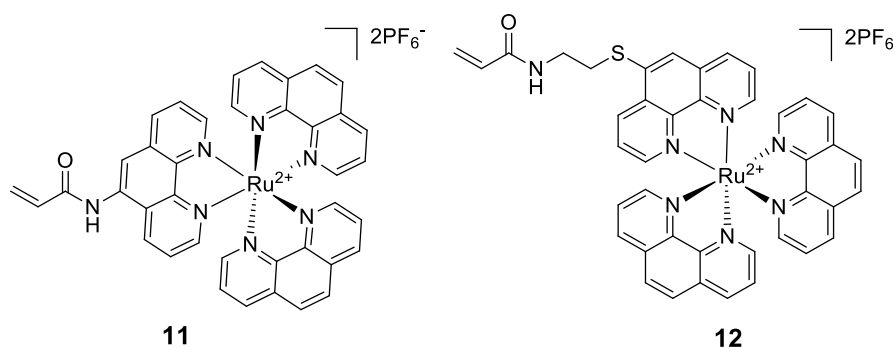


Figure 12: Compounds 11 and 12.

Compound **11** was synthesized in a two-step process (detailed synthetic procedures are provided in Experimental Section in chapters 5.5 and 5.6). First, commercially available 5-amino-1,10-phenanthroline (**10**) was coordinated to the Ru(phen)₂Cl₂ (**5**)

precursor complex under standard conditions, followed by counterion exchange with NH_4PF_6 . The resulting complex was then directly acylated with acryloyl chloride at room temperature under carefully controlled conditions. The product was isolated through liquid-liquid extraction, yielding a red crystalline powder in excellent yields (80-90%). This straightforward synthetic route provided reliable access to the acrylamide-functionalized ruthenium complex.

Compound **12** was prepared through an optimized one-pot procedure using the previously synthesized ligand 2-((1,10-phenanthroline-5-yl)thio)ethanamine (**3**). The synthesis began with ligand coordination to $\text{Ru}(\text{phen})_2\text{Cl}_2$ (**5**) in ethanol, followed by solvent removal and redissolution in dichloromethane. The acylation step was performed *via* careful dropwise addition of acryloyl chloride at 0°C , with subsequent reaction at ambient temperature. This protocol eliminated the need for chromatographic purification through an efficient precipitation process using saturated NH_4PF_6 in ethanol, yielding a high-purity brown powder (detailed synthetic procedures are provided in Experimental Section in chapters 5.5 and 5.6). The successful synthesis was confirmed through ^1H NMR spectroscopy. The spectrum (Figure 13) shows characteristic signals in the aromatic region (δ 7.5-9.0 ppm) corresponding to the phenanthroline ligands, while the acrylamide protons appear as distinct signals at δ 5.5-6.5 ppm.

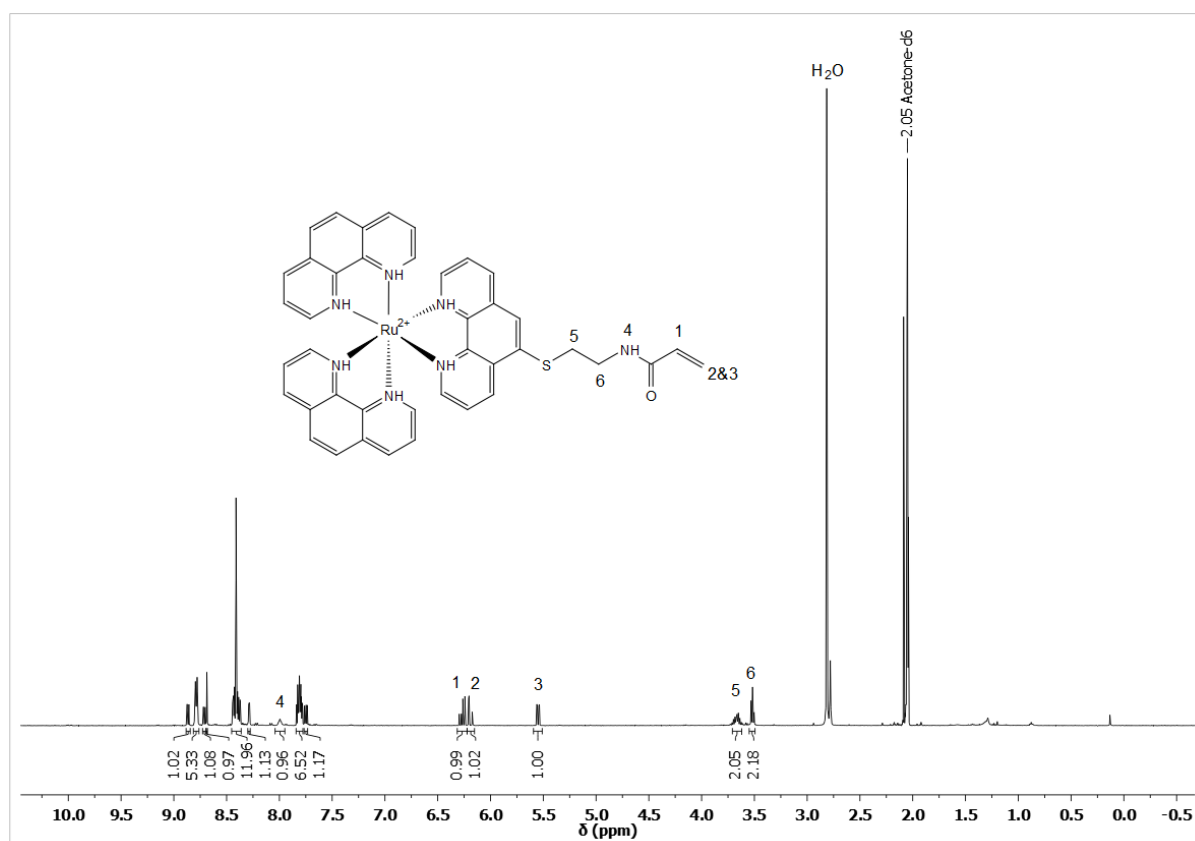


Figure 13: ^1H NMR of complex **12**, showing the desired product.

While compounds **11** and **12** were prepared through sequential steps with isolation of the intermediate products, a more streamlined approach was developed for [ruthenium (1,10-phenanthroline) $_2$ (*N*-(2-((1,10-phenanthrolin-5-yl)amino)-2-oxoethyl)acrylamide)] (PF_6) $_2$, compound **13** as shown in (Figure 14), to minimize purification challenges and improve overall efficiency. The traditional stepwise synthesis had previously resulted in difficult-to-remove impurities, and overall lower yields, especially after the deprotection step, making a one-pot strategy particularly advantageous.

The preparation of compound **13** employed a more sophisticated multi-step one-pot synthesis strategy, beginning with the ligand *tert*-butyl (2-((1,10-phenanthrolin-5-yl)amino)-2-oxoethyl)carbamate (**2**) (Figure 14). The initial complexation was conducted with 1.2 equivalents of ligand **2** with $\text{Ru}(\text{phen})_2\text{Cl}_2$ (**5**) in dry ethanol under argon atmosphere at reflux temperature. A key step in the synthesis was the subsequent Boc deprotection, achieved through the bubbling of HCl gas in a dichloromethane solution over 48 hours at room temperature. This approach proved advantageous as it produced only volatile by-products (2-methylpropene and CO_2), simplifying purification.

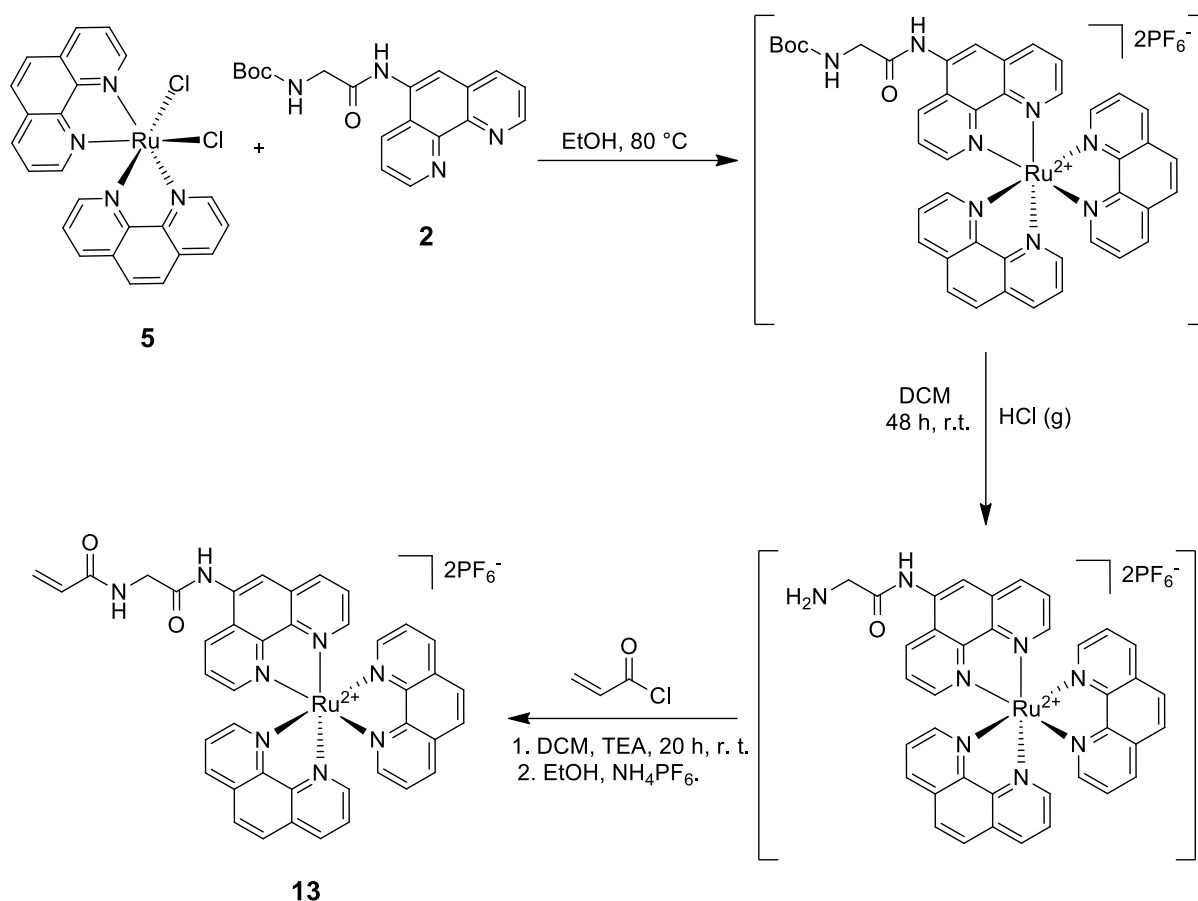


Figure 14: Synthesis of compound **13** in a one-pot synthesis.

The final acylation step was done in multiple steps. The precipitate was dissolved in dichloromethane (DCM) using triethylamine (5 equivalents) as base, followed by controlled addition of acryloyl chloride in DCM at 0°C. The purification process involved solvent removal, vacuum drying, and selective precipitation with saturated NH₄PF₆, followed by centrifugation and ethanol washing, yielding the desired complex as an orange solid with more than 50% overall yield, detailed synthetic procedures are provided in Experimental Section in chapters 5.5 and 5.6.

It is noteworthy that prior to developing the optimized HCl gas deprotection method, trifluoroacetic acid (TFA) was initially investigated as a deprotection reagent. However, this approach proved less efficient as it generated non-volatile salt by-products, necessitating additional purification steps. Furthermore, the final product obtained *via* TFA deprotection exhibited lower purity compared to the product achieved through the HCl gas method.

With the successful synthesis of these ruthenium complexes, the next stage involved their incorporation into polymer systems. However, as would become evident in

subsequent polymerization studies, the incorporation efficiency was critically dependent on the molecular structure of these complexes. These structure-activity relationships will be discussed in the polymer synthesis section.

Phenalenone monomer synthesis

The synthesis of phenalenone monomer **16** was performed in collaboration with Jannik Fryn during his master research under my supervision.

Synthesis of phenalene-1-one

The synthesis of phenalene-1-one (**14**) presented an interesting challenge that was addressed through multiple synthetic attempts, with microwave-assisted synthesis ultimately emerging as the optimal method (method A).[78] This procedure utilized naphthalene and cinnamoyl chloride as starting materials, with AlCl_3 serving as the Lewis acid catalyst, as shown in Figure 15. The reaction was carried out for 12 minutes (100 W, 40 psi, 50 °C) yielding the desired product as a yellow-orange solid with moderate yields (57%).

Three alternative synthetic routes were investigated but proved less effective:

- Method B, conducted at room temperature, failed to produce any desired product.[122]
- Method C, employing conventional heating at 40 °C, resulted in poor yields (10%).[123]
- Method D, using 2-naphthol, glycerine and sodium-3-nitrobenzenesulfonate in $\text{H}_2\text{SO}_4/\text{H}_2\text{O}$ at 140 °C, achieved only moderate yields (30%).[124,125]

The microwave-assisted procedure offered significant advantages beyond just improved yields notably shorter reaction times and enhanced reproducibility. The purification was achieved through column chromatography.

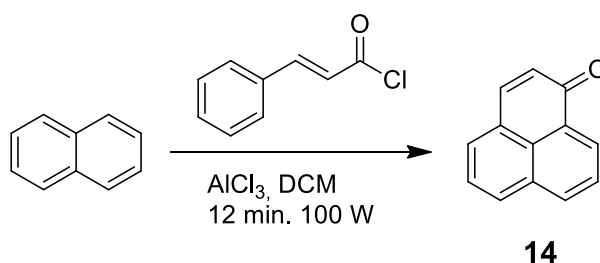


Figure 15: Synthesis of phenalenone (**14**).

Synthesis of 2-(chloromethyl)-1H-phenalen-1-one

Two methods were explored for this transformation. The synthesis involved the reaction of phenalen-1-one (**14**) with paraformaldehyde under acidic conditions (Figure 16). Method A employed a larger solvent volume with an extended reaction time of 3 days, but resulted in poor yields (<5%).^[78]

Method B utilized reduced solvent volumes and a shorter reaction time of 16 hours.^[124] Key modifications included gradual addition of hydrochloric acid (compared to direct addition in method A) and revised extraction procedures. This approach led to significantly improved yields (>25%). The purification protocol was carried out through column chromatography with DCM as single eluent, providing the product as a pure yellow solid.

Based on these results, method B was selected for further synthetic work due to its superior yield and shorter reaction time.

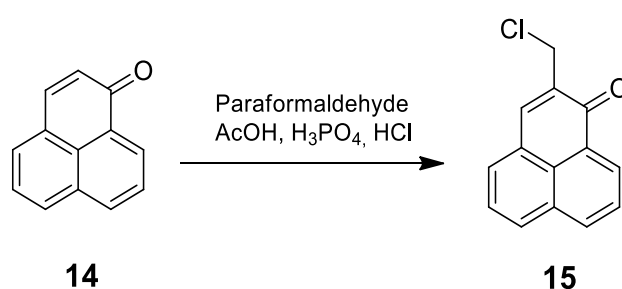


Figure 16: Introducing the chloromethyl functional group to phenalenone.

Synthesis of (1-oxo-1H-phenalen-2-yl)methyl acrylate

The final transformation to the acrylate monomer was explored through three different methods, each employing distinct coupling strategies (Figure 17).

Method A utilized dicyclohexylcarbodiimide (DCC) coupling between the alcohol group of compound **15** and acrylic acid. However, this approach resulted in low yields (<25%) and contamination with dicyclohexylurea (DCU), a byproduct of DCC coupling that is difficult to remove.^[126]

Method B employed sodium acrylate under phase transfer catalysis conditions using Aliquat®336. This method showed modest improvement with yields of approximately 30%, but the product suffered from residual phase transfer catalyst (Aliquat®336) contamination that persisted despite extraction efforts.^[126]

Method C utilized a direct nucleophilic substitution approach with acrylic acid, K_2CO_3 as base, and DMF as solvent at 100 °C for 3 hours.[127] This approach provided superior results with 65% yield.

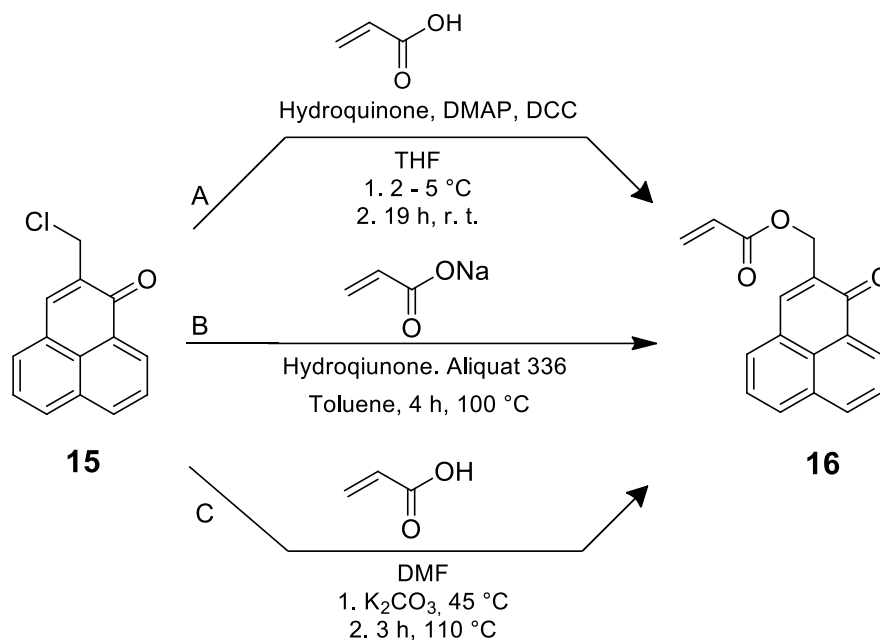


Figure 17: Synthesis methods (A, B and C) of (1-Oxo-1H-phenalen-2-yl)methyl acrylate (**16**).

This final method offered several key advantages including:

- Improved product purity through efficient extraction protocols.
- Higher isolated yields.
- Simplified purification requirements.
- Enhanced reproducibility.
- Shorter reaction times compared to methods A and B.

Based on these results, method C was selected as the preferred synthetic route, providing an efficient pathway to the target monomer (**16**) with good overall yields and high purity.

3.2 Biofilm Disruptors Motifs

Biofilm disruptors represent compounds that target bacterial biofilms through mechanisms distinct from direct antimicrobial action.[128,129] Rather than killing bacteria directly, these agents interfere with biofilm formation and maintenance by disrupting the protective extracellular matrix or inhibiting bacterial adhesion to surfaces.[130,131] When combined with antimicrobial photodynamic agents, biofilm disruptors can potentially enhance therapeutic efficacy by compromising the protective biofilm barrier that typically shields bacteria from treatments.

The selection of biofilm disruptors was based on a comprehensive evaluation of compounds known for their anti-biofilm properties. Four key compounds were selected, which are shown in Figure 18, based on their distinct mechanisms of action and established antimicrobial properties:

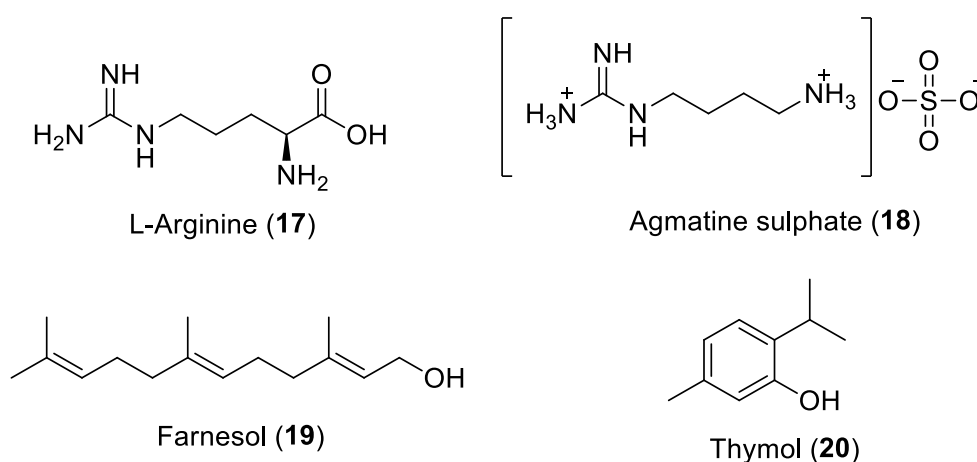


Figure 18: The potential biofilm-disruptors used in this work.

L-arginine (**17**) holds particular significance in antimicrobial applications due to its relationship with guanidinium functional group activity. As a natural amino acid, it shares structural similarities with guanidinium groups, demonstrated by the enhanced cell translocation ability of polyarginine.[128,131,132] This characteristic made L-Arginine particularly valuable for polymer combination studies, as it possesses both the guanidinium functional group and nucleophilic functional groups suitable for incorporation into active ester polymer systems.

Agmatine sulphate (**18**) was selected for its distinctive guanidinium functional group, which plays a crucial role in its antimicrobial activity.[128,132] Its mechanism of action primarily involves interactions with ionic species and lipids present in cell walls and membranes, leading to cell membrane damage through guanidine group

interactions.[128,132] Similar to L-arginine, the compound's dual functionality - containing both guanidinium and nucleophilic groups - made it an ideal candidate for insertion into active ester polymer systems.

Farnesol (**19**) represents a significant antimicrobial compound identified as an active component in various antimicrobial oils.[133] Its mechanism of action differs distinctly from guanidinium-based compounds, primarily functioning through two key pathways: the reduction of polysaccharide production levels in bacterial biofilms and the enhancement of membrane leakage and permeability.[133] This compound was specifically selected for polymer synthesis due to its well-documented antimicrobial properties in its free form.

Thymol (**20**) has been identified as a crucial active component in antimicrobial oils, with its primary mechanism of action centered on the disruption of bacterial quorum sensing.[134–136] This natural compound was chosen for polymer synthesis based on its established antimicrobial properties.

Farnesol (**19**) and thymol (**20**) were converted to their corresponding acrylate monomers to enable their direct incorporation into polymers *via* FRP. The synthesis was accomplished through esterification reactions with acryloyl chloride under mild conditions. These reactions proceeded efficiently, yielding the respective acrylate monomers with yields exceeding 90%. The high conversion efficiency and straightforward purification process made this approach particularly attractive for preparing biofilm disruptor-containing monomers.

The direct polymerization of these biofilm disruptor monomers offered several advantages over post-polymerization modification approaches. By incorporating the biofilm-disrupting units directly during polymerization, a more uniform distribution within the polymer structure could be achieved. This strategy contrasted with the approach used for arginine and agmatine, where post-polymerization modification was preferred due to the presence of their primary amine groups, which provided excellent reactivity with active ester functionalities.

This dual approach to incorporating biofilm disruptors either through direct polymerization of corresponding acrylate monomers or *via* post-polymerization modification provided complementary strategies that expanded the range of bioactive functionalities that could be integrated into the polymeric systems. The selection of the

appropriate incorporation method for each biofilm disruptor was guided by both synthetic considerations and the specific chemical properties of each compound, , which is discussed in detail in the respective synthesis sections below (Sections 3.3.1 and 3.3.2).

3.3 Polymerization Strategies

The development of functional polymers was achieved through two distinct synthetic strategies: PPM and direct FRP, with both approaches proving successful in their implementation, each method offered unique advantages and faced specific limitations as discussed in more detail further below. Summary of both strategies is demonstrated in Figure 19.

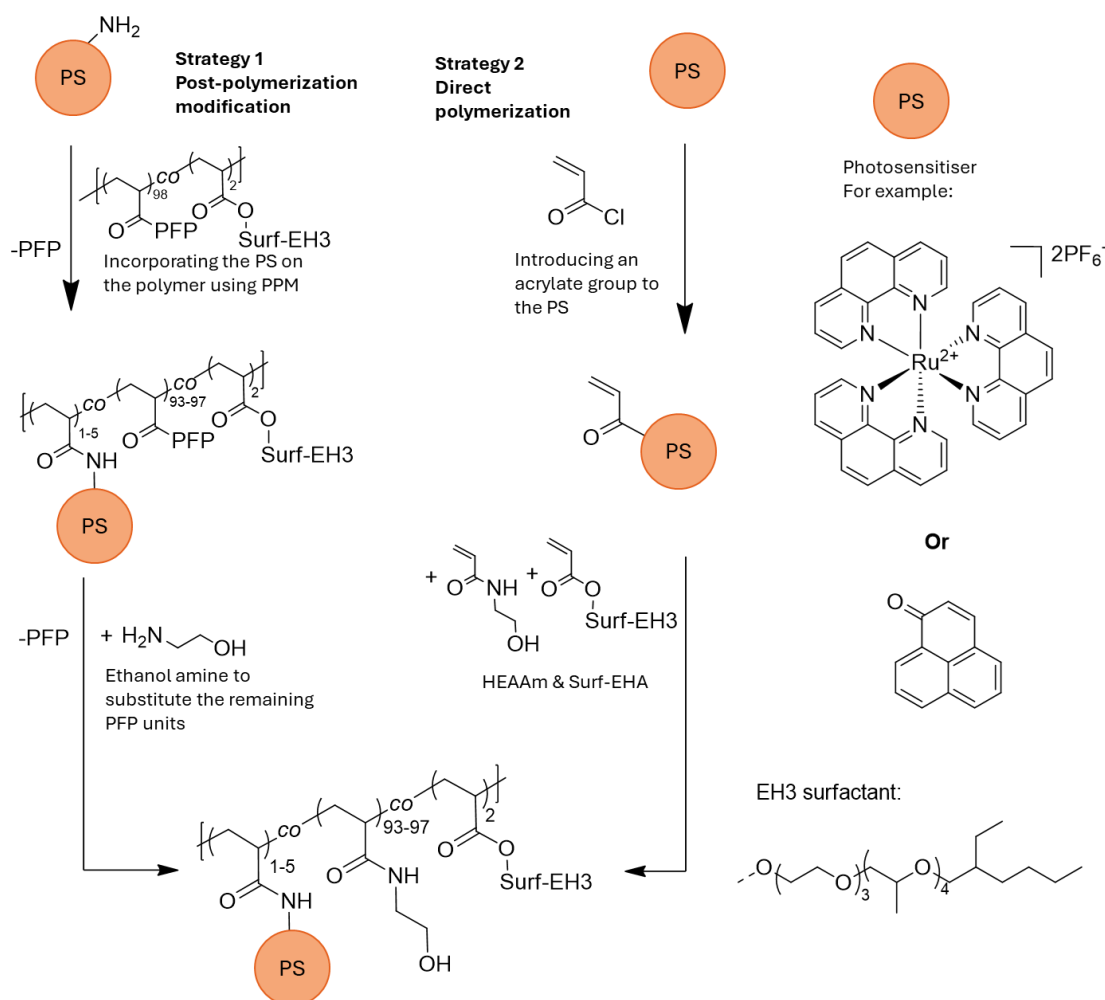


Figure 19: Summary of the polymer synthesis strategies explored in this thesis. Post-polymerization modification (PPM) and free radical polymerization (FRP) were used to obtain the desired polymers carrying the photosensitizer (PS).

PPM demonstrated versatility through its ability to incorporate various molecular fragments containing appropriate functional groups, such as amines and hydroxyls, in an efficient one-pot synthesis approach. This flexibility in molecular incorporation represents a significant advantage for tailoring polymer properties. Conversely, direct FRP offered a more streamlined synthetic route by constructing the polymer chain

directly from pre-functionalized monomers, eliminating the need for additional modification steps and thereby enhancing overall process efficiency.

However, both strategies presented distinct challenges. PPM approach, while versatile, introduced additional complexity to the synthetic process. The requirement for multiple reaction steps potentially led to lower overall yields and increased the possibility of incomplete modifications. Direct FRP, while offering a simpler synthetic route, required all functional components to be incorporated during the polymerization step. This approach demands that all comonomers possess compatible reactivity ratios to ensure effective copolymerization and achieve the desired polymer composition. Unlike PPM, where modifications can be made after polymerization, the final polymer properties in FRP are determined by the copolymerization behavior of the selected monomers.

3.3.1 Post-Polymerization Modification (PPM)

Synthesis of reactive polymers.

The development of suitable reactive polymers for PPM required careful selection of an active ester monomer that would provide both high reactivity and good stability during modifications.

While several candidates, including pentafluorophenyl 4-vinylbenzene sulfonate (PFP4VBS, **22**) and *N*-hydroxysuccinimide acrylate (NHSA, **23**), were evaluated, pentafluorophenyl acrylate (PFPA, **21**) emerged as the optimal choice based on several key advantages, the active esters are shown in Figure 20.

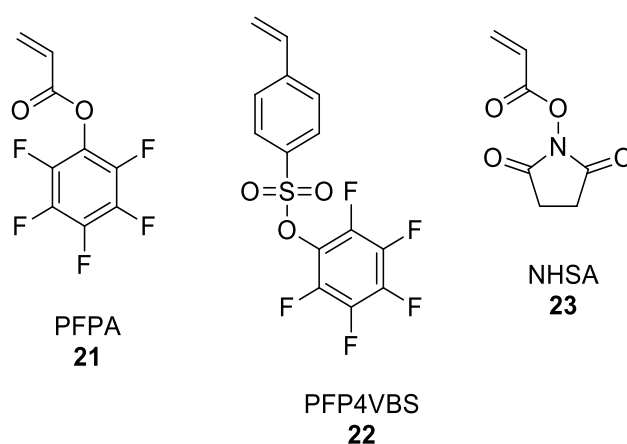


Figure 20: Active esters, pentafluorophenyl acrylate (PFPA, **21**), pentafluorophenyl 4-vinylbenzene sulfonate (PFP4VBS, **22**) and *N*-hydroxysuccinimide acrylate (NHSA, **23**), synthesized as monomers and (co)polymerized in a subsequent step.

The selection of PFPA was driven by its superior characteristics: high reactivity towards primary alkyl amines, excellent hydrolytic stability, and broad solubility across various solvents. To enhance the properties of the resulting polymers, PFPA was copolymerized with a surfactant monomer (Surf-EH3A, 5.4), which was incorporated to introduce hydrophilic-hydrophobic balance and amphiphilicity to the system (Figure 21).[137]

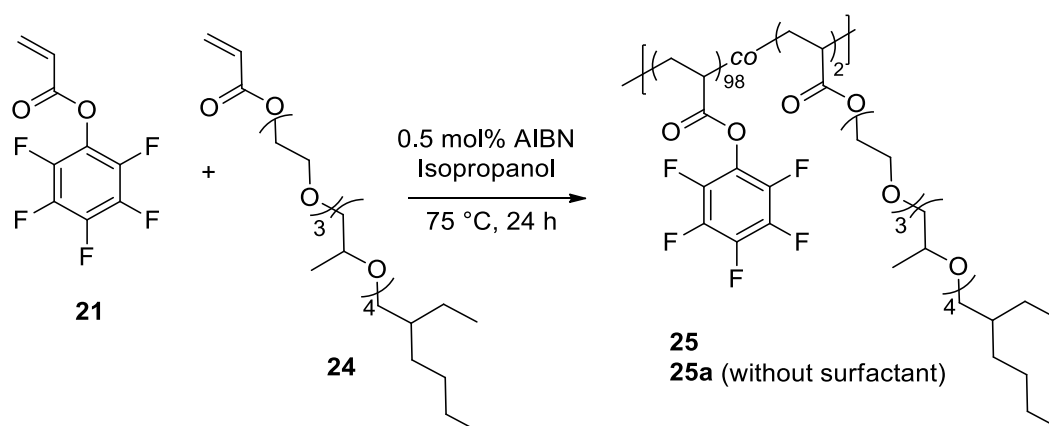


Figure 21: Synthesis of polyPFPA₉₈-co-Surf-EH3A₂ (**25**) and polyPFPA (**25a**).

Alternative active esters were thoroughly evaluated but presented various limitations. NHSA-based polymers, while successfully synthesized, exhibited restricted solubility, significantly limiting the choice of reaction solvents for subsequent modifications. PFP4VBS, despite showing high reactivity toward both aliphatic and some aromatic amines, presented synthetic challenges. Its preparation required a two-step process involving toxic reagents such as thionyl chloride. Furthermore, the presence of aromatic protons in PFP4VBS complicated the NMR analysis of subsequent polymers, particularly when incorporating compounds containing aromatic groups like ruthenium complexes.

The polymerization of these active esters was accomplished through either RAFT polymerization or FRP. Characterization of the resulting polymers was achieved through a combination of ¹H NMR, ¹⁹F NMR, and GPC analyses. The ¹H NMR provided clear structural confirmation, while ¹⁹F NMR proved particularly valuable for monitoring the conversion and reactivity of the PFP groups. The absence of ¹⁹F NMR signals in the final modified polymers confirmed complete conversion of the active ester groups.

Building on the strategic design introduced earlier, initial unsuccessful attempts to modify polymers using aromatic amine-containing compounds like 5-amino-1,10-phenanthroline confirmed the critical importance of alkyl spacer incorporation. Despite various approaches, only marginal reactivity (approximately 5% grafting yield) was achieved, even when using two-fold excess of reagents, the reactions are shown in Figure 22. This underscored the necessity of the alkyl spacer strategy, which would prove crucial in subsequent polymer modification reactions.

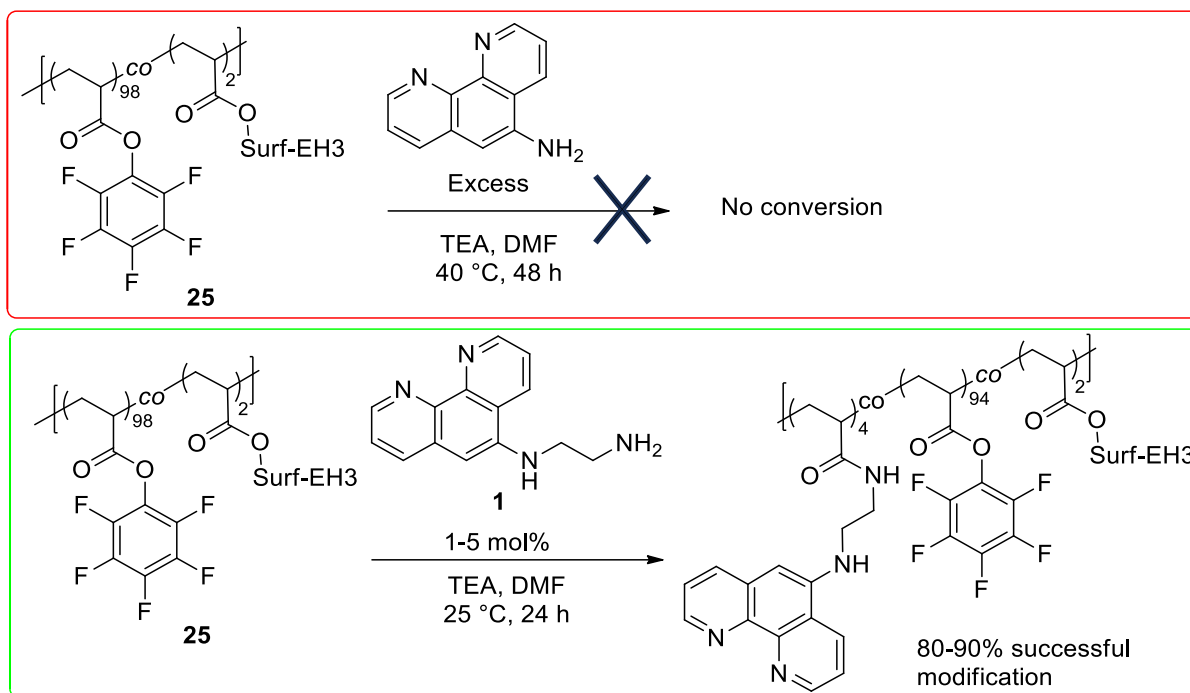


Figure 22: PPM reaction using an aromatic amine compound (top) and an aliphatic primary amine-containing compound (bottom).

PPM reactions to introduce aPDT units.

The pre-made aPDT units, equipped with the aliphatic amine group, were incorporated into the reactive polymer *via* PPM reaction. The modification process was conducted using a sequential one-pot approach, beginning with the dissolution of the reactive polymer in DMF. Different aPDT units were successfully incorporated, including both the ruthenium complexes as inorganic aPDT/PS and phenalenone as organic aPDT/PS.

The synthetic procedure involved careful control of reagent addition and stoichiometry. A specific quantity of aPDT units (typically 1-2 mol% relative to polymer repeating units) was first added to the reactive polymer solution. Following this initial modification, ethanolamine was introduced to quench the remaining active ester groups, ensuring complete modification of the polymer. This strategy resulted in polymers containing approximately 1-2% PDT units, 2% surfactant (where applicable), with the majority (~96%) consisting of *N*-hydroxyethyl acrylamide (HEAAm) units resulting from ethanolamine addition.

The choice of HEAAm as the major component was strategic, as these polymers are well-documented for their biocompatibility, antifouling properties, and hydrophilicity, making them particularly suitable for biomedical applications (as discussed in section 1.3 Polymer-based Drug Delivery Systems).

Several successful modifications of the reactive polymer were achieved using different PDT units. The incorporation of ruthenium complexes was initially performed at room temperature in DMF, using triethylamine as a base. Following the addition of the ruthenium complex and an appropriate reaction time, the remaining PFP units were modified with ethanolamine to yield a HEAAm-based polymer. The successful incorporation was confirmed through NMR spectroscopy and UV-visible absorption spectroscopy, with the latter showing the characteristic absorption band at 450 nm.

Polymer **26** was synthesized using a RAFT-polymerized active ester precursor (polyPFPA₉₈-co-Surf-EH3A₂)-CTA provided by Karla Heckhäuser during her lab course (Figure 23). This precursor polymer retained the trithiocarbonate chain-end from the RAFT agent, which remained present in the final modified polymer and may influence its biological behavior. The modification proceeded at room temperature with ruthenium complex 6, followed by ethanolamine quenching. Partial hydrolysis of PFP

units was observed, yielding free carboxylic acid groups that impart negative charge to the polymer in aqueous media.

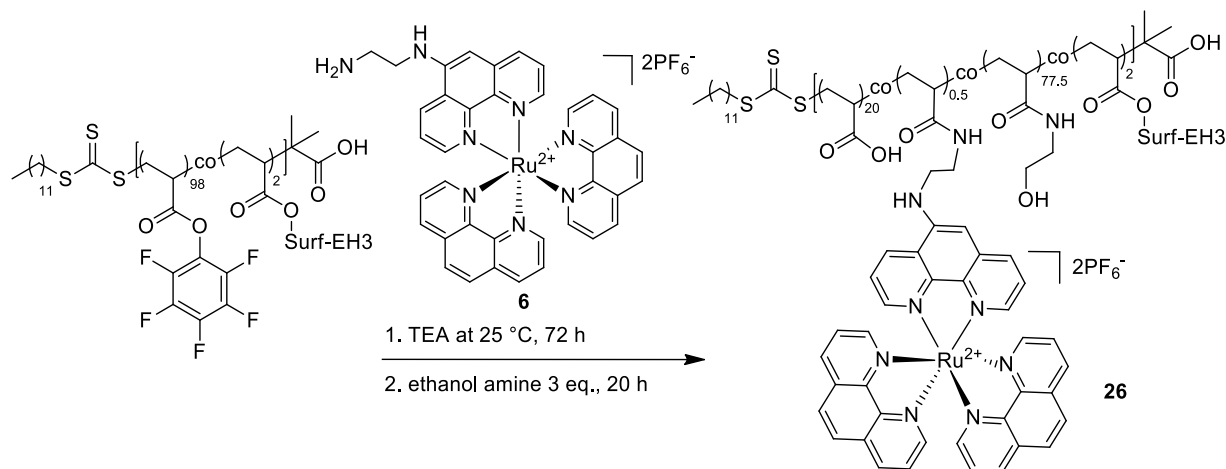


Figure 23: Synthesis of polymer **26**. The trithiocarbonate RAFT chain-end remains in the final polymer. Partial hydrolysis of pentafluorophenyl ester units resulted in carboxylic acid formation.

Polymer **27** was synthesized using the FRP-polymerized active ester precursor **25**, (Figure 24). The modification with ruthenium complex **8** and subsequent ethanolamine treatment proceeded similarly. Partial hydrolysis was also observed here similar to polymer **26**.

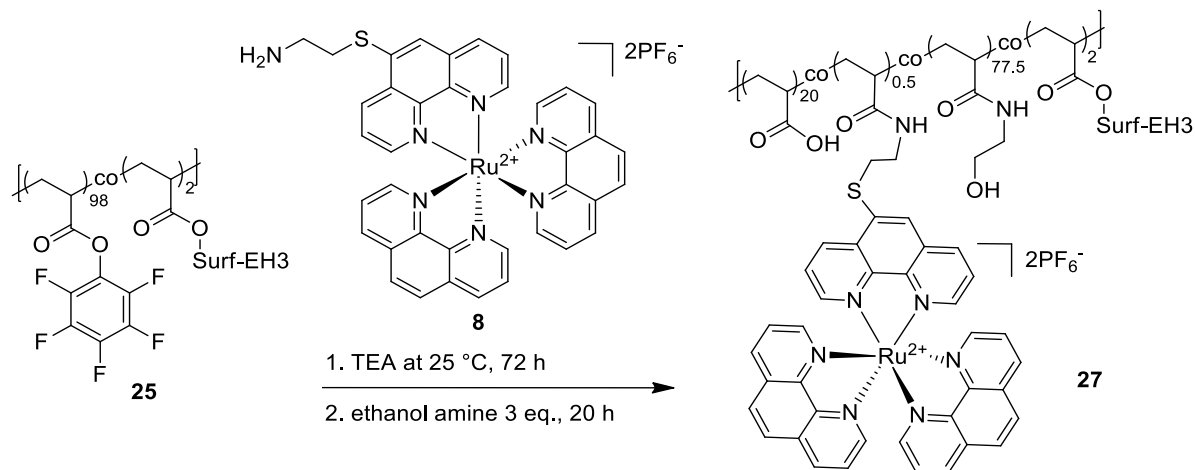


Figure 24: Synthesis of polymer **27**.

This hydrolysis was particularly pronounced in reactions performed at temperatures higher than 25°C, details shown in Figure 25. Various factors were identified as potential causes for this undesired hydrolysis, including impurities, humidity, and insufficiently dried solvents. This challenge was subsequently addressed by using rigorously dried solvents and maintaining an inert atmosphere during the reaction, as detailed in Figure 26.

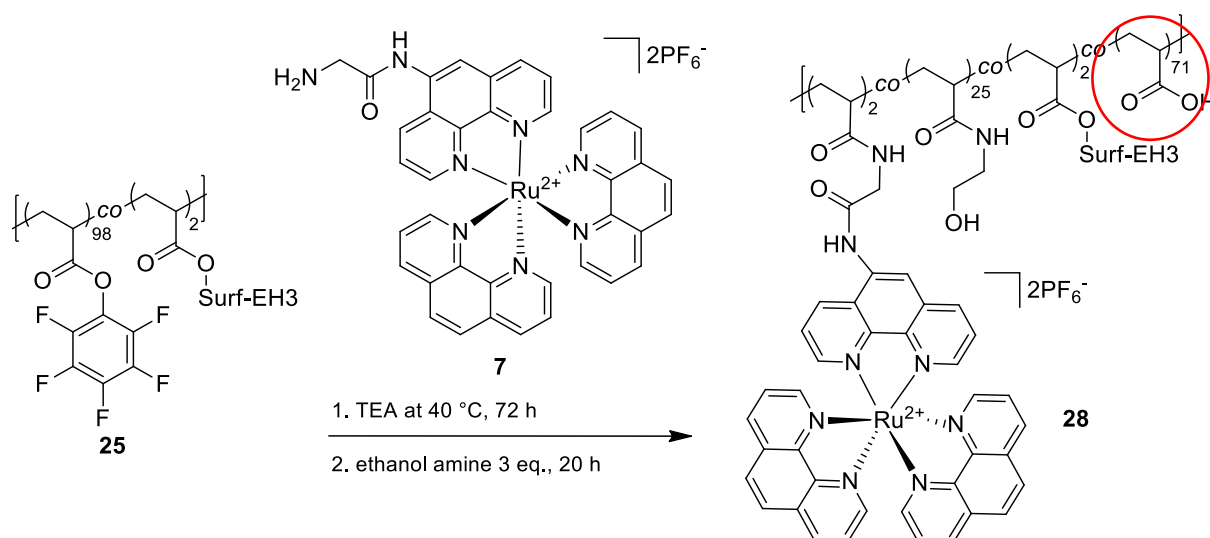


Figure 25: PPM reaction at 40 °C in the first step for introducing the ruthenium complex **7** and at room temperature in the second step for modifying the remaining PFP units with ethanol amine. Heating the reaction for longer time and using undried solvents resulted in more hydrolysis (carboxylic acid units in the red circle).

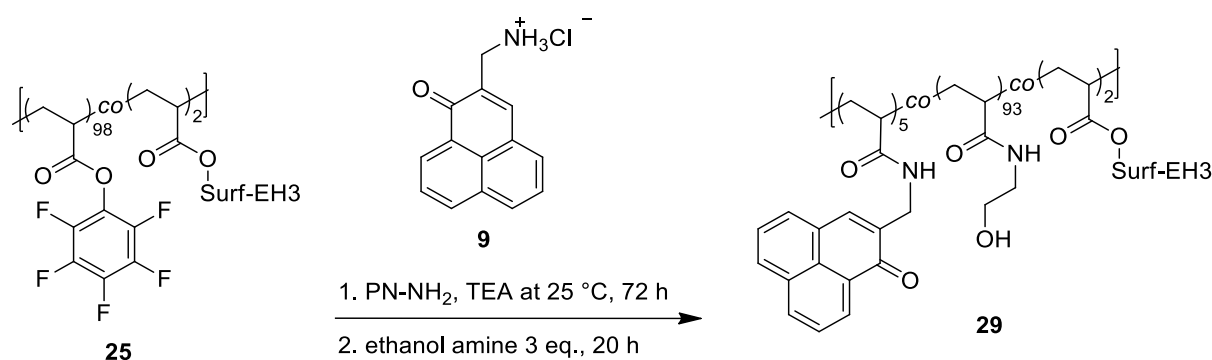


Figure 26: PPM reaction at room temperature. First compound **9** was added and after 24 hours, the remaining PFP were modified with ethanol amine. The solvents and materials used were dried and purified in advance to minimize the possibility of hydrolysis of the active ester units.

The extent of hydrolysis was quantitatively determined through ¹H NMR analysis by examining the correlation between backbone protons and the ethyl group protons of HEAAm units. In an ideal case with complete conversion to HEAAm (97-98 mol% of the polymer), a ratio of three backbone protons to four ethyl group protons (of HEAAm) should be observed. However, for the polymer synthesized at 40 °C under non-optimized conditions, the ¹H NMR showed only one ethyl group proton for every three backbone protons and the acid proton. This significant deviation from the expected ratio indicated substantial hydrolysis to carboxylic acid groups, which contribute only backbone protons. This analysis provided an estimation of the HEAAm incorporation and, by extension, the degree of hydrolysis in the polymer. ¹H NMR of a polymer, in which the hydrolysis was minimized, is depicted in Figure 27.

^{19}F NMR proved particularly valuable in monitoring the reaction progress, as the complete disappearance of fluorine signals indicated successful modification of all PFP units (e. g. Fig. SI 36 in Appendix). GPC and UV-Vis absorption spectroscopy provided additional confirmation of the polymer structure and incorporation of aPDT units.

The hydrolysis reaction, while initially undesired, led to an interesting result in the biological tests, as the polymers possessed a negative charge, which was confirmed by electrophoresis tests (detailed further in the biological evaluation chapter, section 3.4.1). This charge characteristic would later prove significant in influencing the biological activity of the polymers.

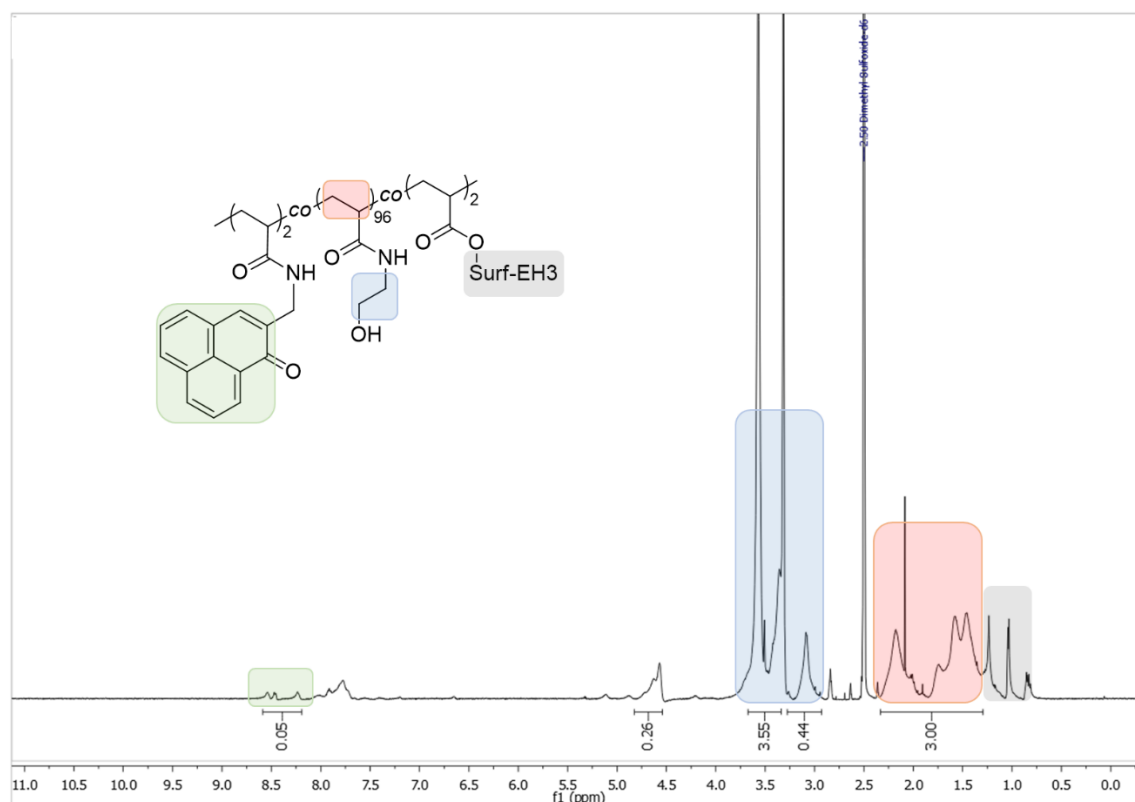


Figure 27: ^1H NMR of polyPN₂-co-HEAAm₉₆-co-Surf-EH3A₂ (**29**). The hydrolysis was minimized in this case, and this was observed through the correlation between backbone protons (indicated in red) and the ethyl group protons of HEAAm (indicated in blue). ^1H NMR of a polymer, in which it's partially hydrolyzed, is depicted in Fig. SI 33 in the Appendix.

PPM reactions to introduce biofilm disruptors

The synthesis of the following polymers was performed in collaboration with Tristan Zemke during his master research under my supervision.

The selection of agmatine and L-arginine for PPM was based on their molecular characteristics. Their primary amine groups provided excellent reactivity with the active ester groups of the polymer backbone, while their guanidinium functionalities offered complementary antimicrobial properties through different mechanisms.[128,132]

The combination of nucleophilic groups for polymer attachment and guanidinium groups for bacterial interaction made these compounds particularly suitable for the PPM approach. Furthermore, their water solubility and stability under the modification conditions supported efficient polymer functionalization, whereas the more hydrophobic biofilm disruptors (thymol and farnesol) were better suited for direct polymerization approaches (0).

PPM of polyPFPA-co-SurfEH3A (**25**) was successfully achieved using both L-arginine (**17**) and agmatine sulfate (**18**) under mild conditions. The modification reactions were conducted in DMF using triethylamine as base and DMAP as catalyst, with the nucleophilic substitution proceeding at room temperature for 24-48 hours (detailed synthetic procedures are provided in Section 5.8.8 of the experimental chapter). Complete functionalization of the polymer was ensured through a secondary modification step using ethanolamine, which reacted with the remaining pentafluorophenyl ester groups. The modified polymers were effectively purified through a combination of precipitation in ethyl acetate and liquid-solid extraction, followed by dialysis against water for biological testing applications. The same process was carried out using polyPFPA (**25a**). The target polymers are illustrated in Figure 28.

Addition of DMAP as a nucleophilic catalyst proved crucial, significantly improving the reaction yields compared to previous modifications where it was not employed. This optimization highlights the importance of catalyst selection in achieving efficient polymer modification.

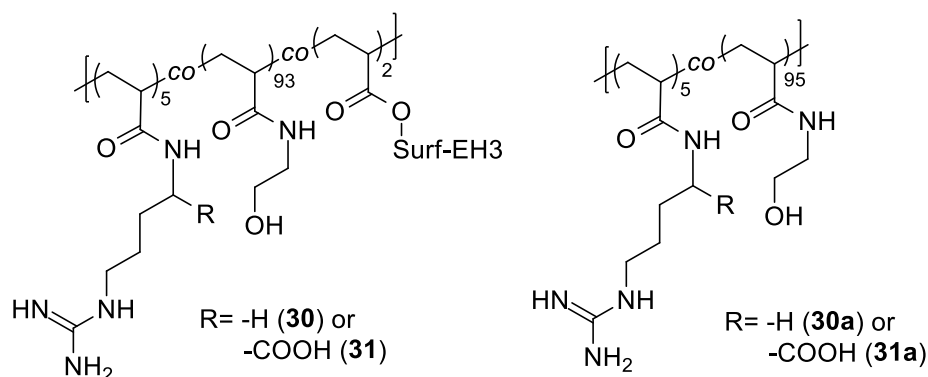


Figure 28: Introducing L-arginine (**17**) or agmatine (**18**) to the polymers **25** & **25a** by PPM.

In an effort to create polymers with higher antimicrobial activity, a strategy was developed to simultaneously incorporate both ruthenium complex and L-arginine onto the polymer backbone.

Simultaneous incorporation of both photosensitizer and biofilm disruptor units aimed to create multifunctional materials that could target bacterial infections through complementary mechanisms. This one-pot modification approach would potentially simplify the synthetic process while ensuring both functionalities were present in the final polymer (Figure 29).

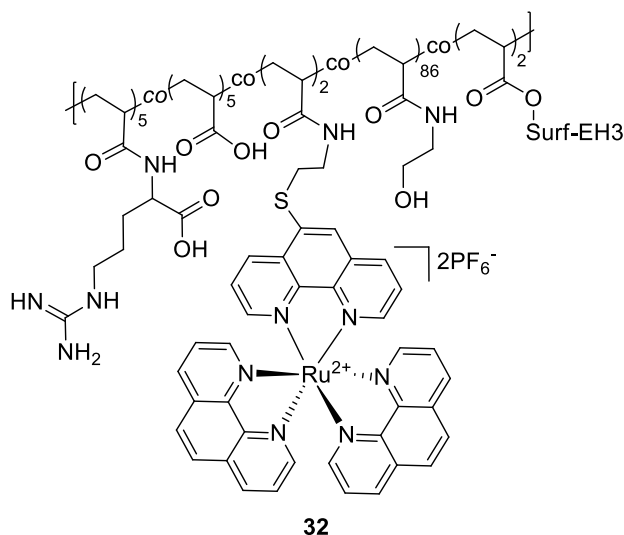


Figure 29: PolyL-arginin₅-co-AA₅-co-Ru(S)₂-co-HEAAm₈₆-co-Surf-EH3A₂ (**32**). The polymer was made in a one pot synthesis adding the different amine derivatives for PFP substitution one after the other. The used solvent was dried in advance, however the polymer got partially hydrolyzed probably from humidity because of multiple addition of the starting materials which probably introduced humidity from air to the reaction vessel. Additionally, the long reaction time might have aggravated this.

This parallel modification approach presented analytical challenges, particularly in quantitative analysis. While ^1H NMR spectroscopy showed significant signal overlap in many regions, a characteristic L-arginine proton signal remained distinguishable, confirming successful incorporation despite the peak overlap.

Notably, the extent of hydrolysis was observed to be significantly reduced compared to previous reactions, as evidenced by spectroscopic analysis. This improved stability was likely due to the optimized reaction conditions developed through earlier experiments, including the use of dry solvents and careful control of reaction temperature

Failed PPM reactions

An alternative approach to PPM reactions was investigated, where the strategy involved first grafting the phenanthroline ligand onto the polymer, followed by complex formation with $\text{Ru}(\text{phen})_2\text{Cl}_2$ (**5**) directly on the polymer backbone (detailed synthetic procedures are provided in Section 5.10.2 of the experimental chapter).

The goal of this stepwise approach was to build ruthenium complexes directly on the polymer backbone, potentially offering a more efficient synthetic route. After successfully grafting the ligand, the pre-complex $\text{Ru}(\text{phen})_2\text{Cl}_2$ (**5**) was introduced to complete the complex formation using the pre-grafted ligand, as shown in Figure 30. Despite multiple attempts with variations in reaction conditions and reactant ratios, the reaction consistently failed to produce the desired result.

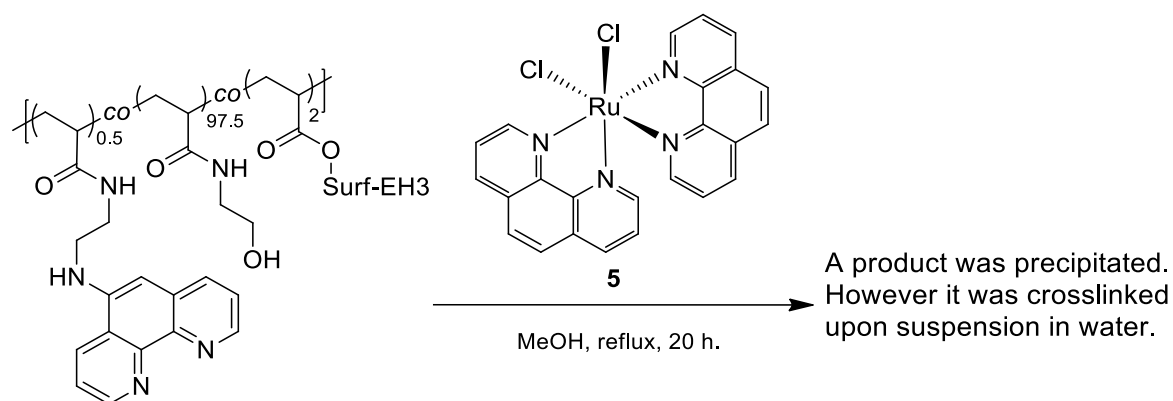


Figure 30: Failed attempt to build a ruthenium complex on the polymer.

In several cases, the material precipitated during the reaction process, and the resulting polymer became insoluble in both organic and aqueous solvents. The most probable explanation for this outcome is unintended crosslinking of the polymer, where free or excess ruthenium species potentially coordinated to phenanthroline units on multiple polymer chains, creating intermolecular bonds. These bonds likely caused the polymer to lose its solubility and become a rigid, precipitated material.

This outcome demonstrated the need for more refined synthetic strategies and suggested that direct complex formation on the polymer might require alternative approaches to achieve successful integration.

3.3.2 Polymers Synthesis via Free Radical Polymerization (FRP)

Free radical polymerization was employed to directly incorporate photosensitizer monomers into the polymer backbone alongside HEAAm and Surf-EH3A comonomers. Polymerizations were conducted using AIBN (0.5 mol%) as initiator at 65°C under argon atmosphere for 24 hours.

The selection of an appropriate polymerization solvent presented a significant challenge due to the diverse solubility requirements of the monomers. Methanol proved suboptimal due to limited solubility of ruthenium complex monomers, while acetone led to premature polymer precipitation.

DMSO was selected as the preferred solvent despite its high boiling point, as it provided good solubility for all monomers. Polymer isolation was managed by maintaining minimal solvent volumes and precipitation in appropriate non-solvents, followed by dialysis purification.

The synthesized polymers were characterized by ^1H NMR spectroscopy for composition analysis, GPC for molar mass determination, and UV-Vis absorption spectroscopy to confirm photosensitizer incorporation.

aPDT polymers made by free radical copolymerization

The aPDT monomers (ruthenium complex and phenalenone) were copolymerized with two key comonomers: *N*-hydroxyethyl acrylamide (HEAAM) and Surf-EH3A. The amphiphilic nature of the surfactant monomer and the hydrophilic character of HEAAM were designed to enhance the polymer's interaction with biological systems, Figure 31 shows some examples of the obtained polymers through this strategy.

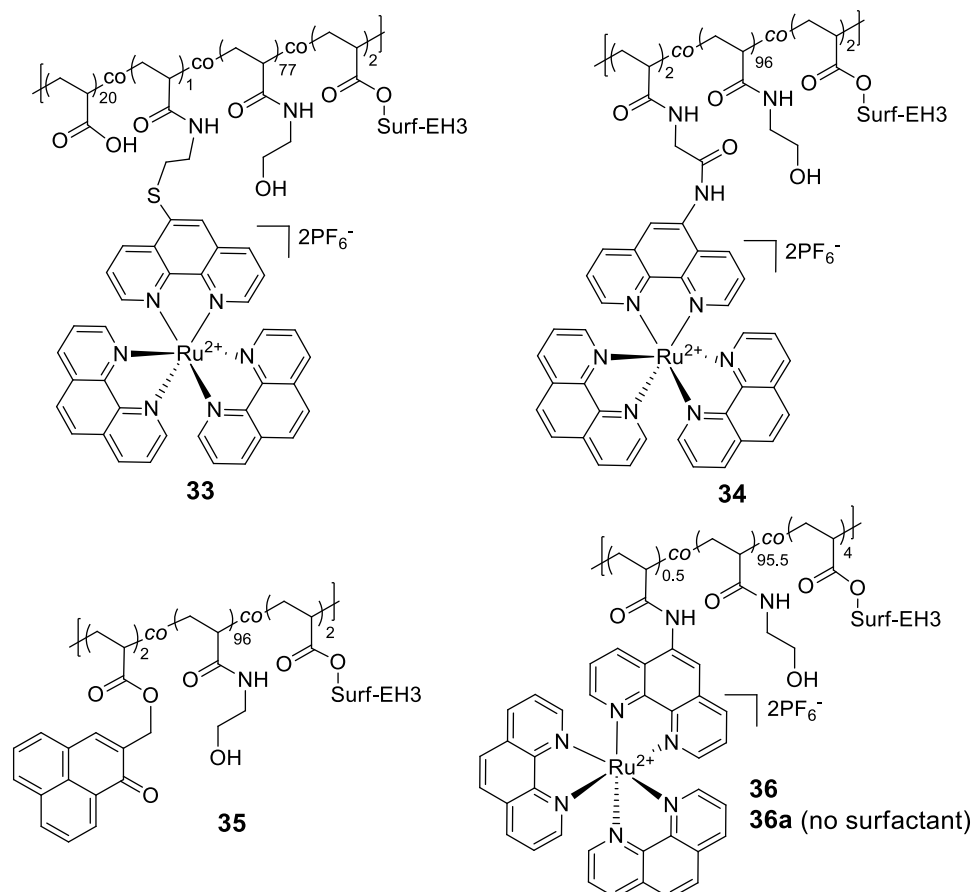


Figure 31: Copolymers synthesized by strategy 2 (FRP).

Initial characterization of the complex-containing polymers through electrophoresis (details are found in section 3.4.1, page 81) revealed that the synthesized copolymers possessed a slight positive charge, attributed to the presence of the ruthenium complex within the polymer structure. To expand the range of available materials and investigate charge-dependent effects, negatively charged variants were also synthesized by incorporating acrylic acid as a comonomer. The polymer charge proved to be a critical factor in subsequent biological testing, as detailed extensively in the biological evaluation chapter.

Following the successful incorporation of the ruthenium complex, phenalenone was also successfully incorporated as an alternative aPDT monomer using the same copolymerization strategy with HEAAm and Surf-EH3A. The resulting polymer displayed similar characteristics to its ruthenium-containing counterpart, demonstrating the versatility of the synthetic approach.

Building on these achievements, a novel polymer containing both photosensitizers (ruthenium complex and phenalenone) was successfully synthesized. The presence of both aPDT moieties was confirmed through comprehensive analysis using ^1H NMR and UV-Vis absorption spectroscopy, (as shown in Figure 32), which displayed the characteristic signals and absorption patterns of both photosensitizers within the polymer structure.

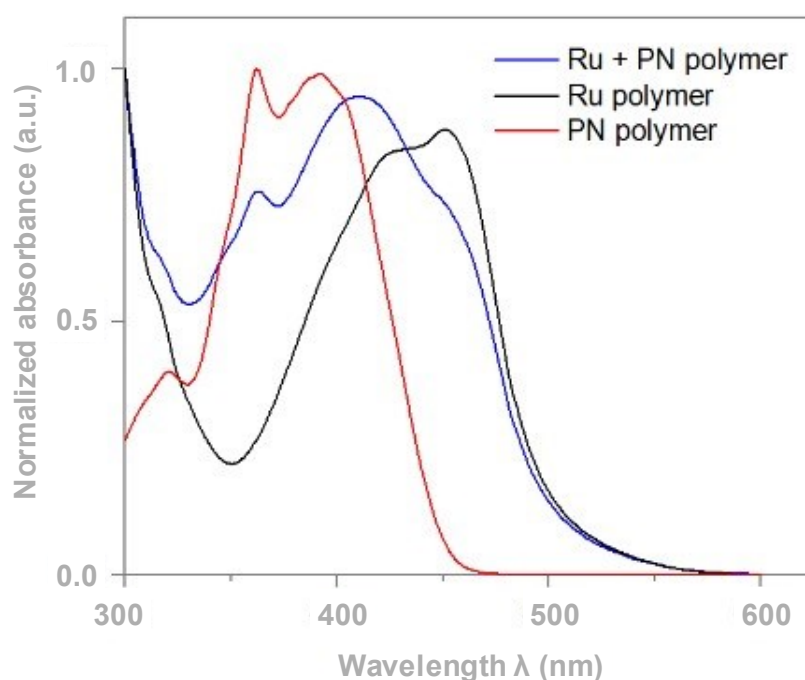


Figure 32: UV-Vis absorption of three polymers, (black) which is a copolymer containing ruthenium complex (**36**), (red) copolymer containing phenalenone (**35**), (blue) copolymer containing ruthenium complex and phenalenone. All polymers contain HEAAm as the main component and also Surf-EH3A.

The selection of an appropriate polymerization solvent presented a significant challenge due to the diverse solubility requirements of the various monomers. Through systematic evaluation of different solvents, methanol was initially tested but proved suboptimal due to the limited solubility of the ruthenium complex. Attempts with acetone were similarly challenging, as the forming polymer showed premature precipitation during the reaction process. After careful consideration, DMSO was

selected as the preferred solvent despite its high boiling point, which could potentially complicate polymer isolation through precipitation, particularly in dilute systems. This challenge was effectively managed by maintaining minimal solvent volumes, ultimately leading to successful polymerization.

The synthesized polymers underwent comprehensive characterization using multiple analytical techniques. ^1H NMR spectroscopy provided structural confirmation and enabled composition analysis, particularly through the characteristic signals of both the photosensitizer units and polymer backbone (e. g. Figure 33 of polymer **37**). GPC analysis revealed the molar mass distributions, while UV-Vis absorption spectroscopy confirmed the successful incorporation of the photosensitizer units through their characteristic absorption bands.

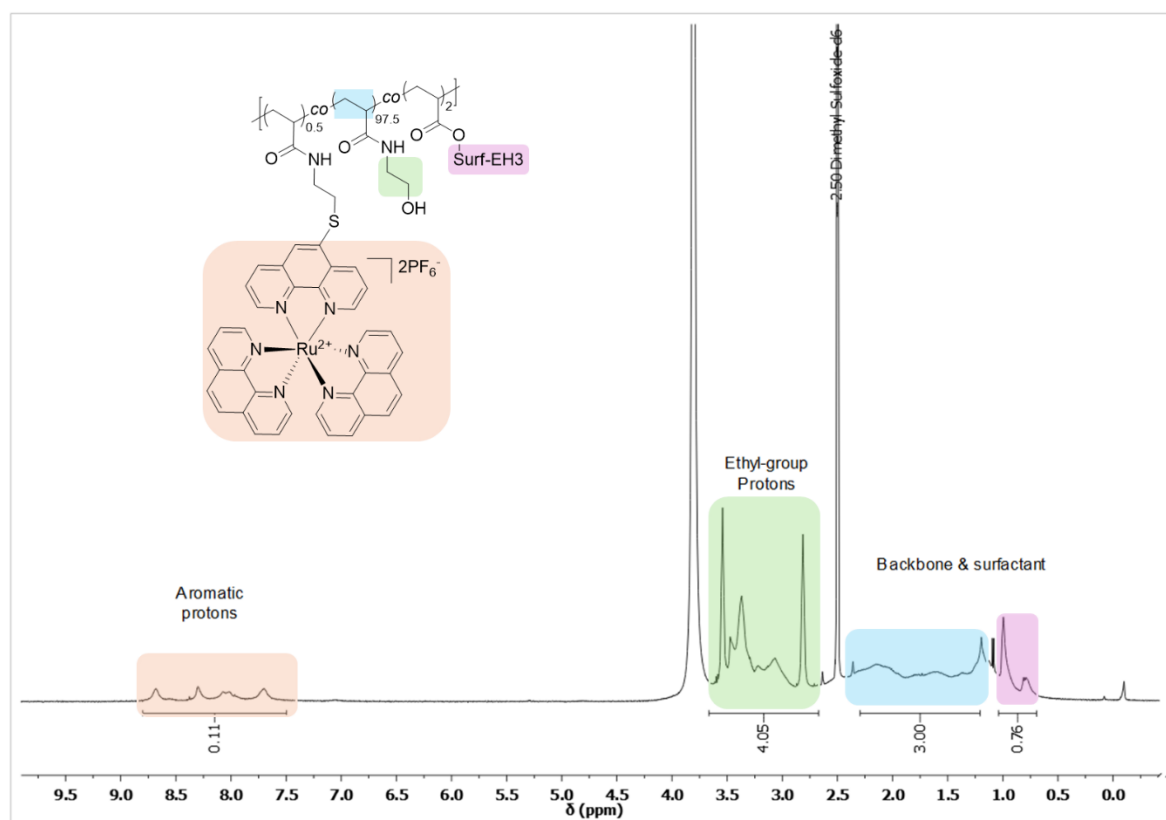


Figure 33: ^1H NMR of polyRu(S)_{0.5}-co-HEAAm_{97.5}-co-Surf-EH3A₂ (**37**). The aromatic protons of the ruthenium complex are clearly visible between 7.5-9 ppm. Ethyl group protons of HEAAm as well as the protons of the backbone and the surfactant are distinguishable in the aliphatic region.

Polymers containing aPDT units and biofilm disruptors

The development of polymers containing both aPDT units and biofilm disruptors represented a strategic approach to enhance antimicrobial efficacy. The underlying hypothesis was that biofilm disruptors could potentially improve the effectiveness of aPDT by compromising the protective biofilm matrix surrounding bacterial communities. This synergistic design aimed to address one of the major challenges in antimicrobial therapy, which is the reduced susceptibility of biofilm-encased bacteria to treatment.

The rationale behind this dual-functionality approach was based on the complementary mechanisms of action. While aPDT units generate reactive oxygen species to induce bacterial cell death upon light activation, biofilm disruptors could simultaneously weaken the extracellular polymeric substances (EPS) that form the protective biofilm matrix. This combination was hypothesized to enhance penetration of both the photosensitizer and light into the bacterial community, potentially leading to more effective bacterial eradication.

Thymol and farnesol were successfully incorporated independently into the polymer structure alongside the aPDT units using free radical polymerization (Figure 34). The selection of these specific biofilm disruptors was based on their distinct mechanisms of biofilm disruption, thymol's ability to interfere with bacterial quorum sensing and farnesol's capacity to reduce polysaccharide production in biofilms, as discussed with the monomer synthesis above.[134] These properties were expected to complement the photodynamic action of the aPDT units. The incorporation of the substances was confirmed through ^1H NMR analysis (e. g. Figure 35).

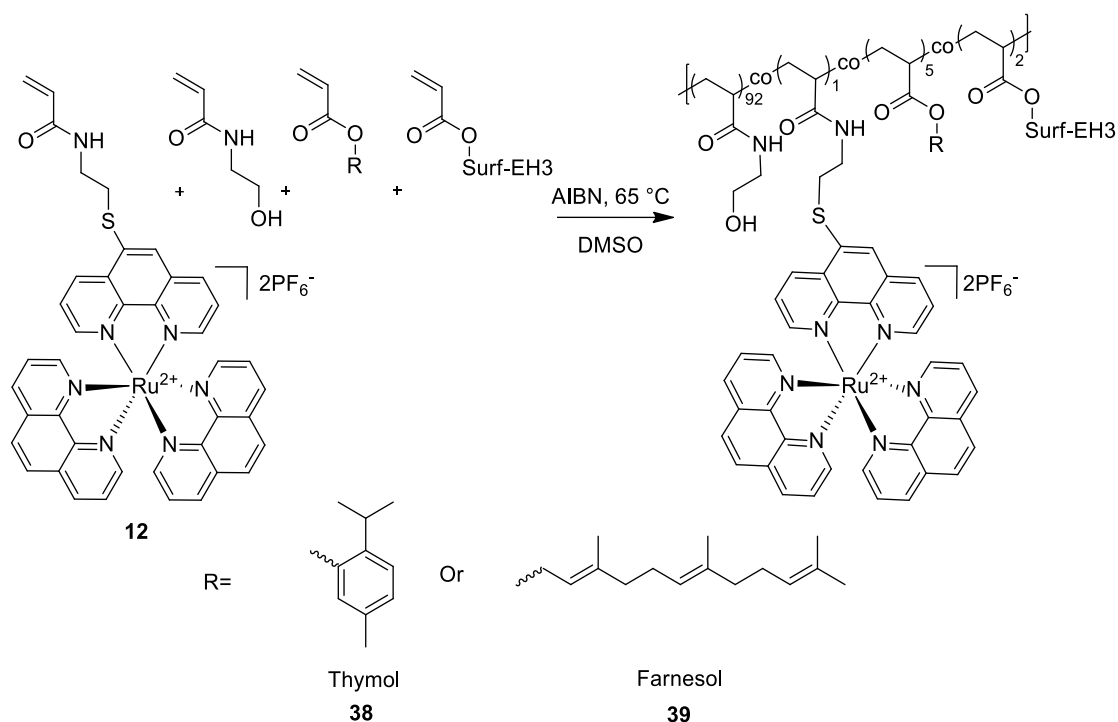


Figure 34: Synthesis of copolymers **38** & **39** containing aPDT units and biofilm disruptors (thymol and farnesol).

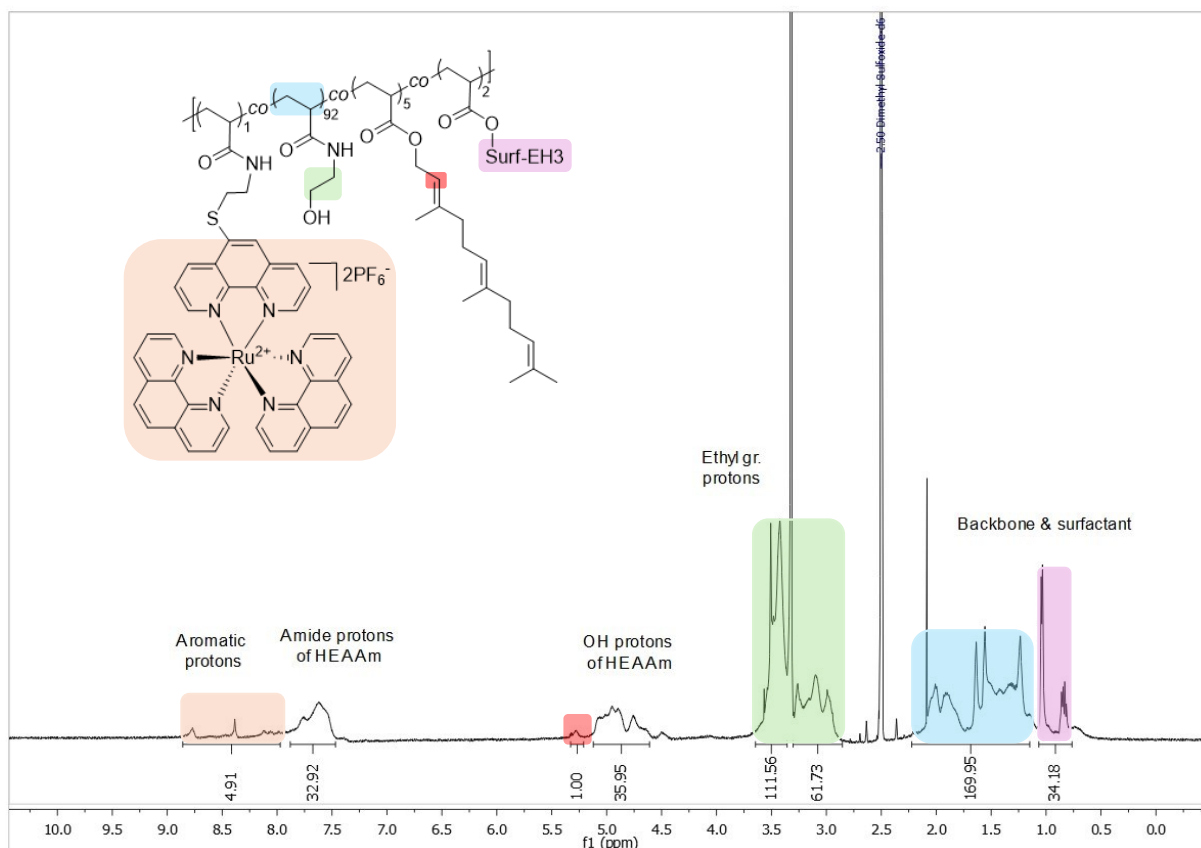


Figure 35: ¹H NMR of polyRu(S)₁-co-HEAAm₉₂-co-Far₅-co-Surf-EH3A₂ (**39**). The protons of the grafted substances are clearly observed especially of the Ru(S). Farnesol protons overlap with other signals however one signal was distinguishable.

Polymers containing biofilm disruptors, HEAAm & surfactant (no aPDT)

The synthesis of polymers containing only biofilm-disrupting units was undertaken to establish a comprehensive understanding of their independent activity in the absence of photodynamic therapy. This approach was designed to provide reference materials for comparing the efficacy of combination therapies versus single-mode treatments, helping to elucidate whether any observed effects in the dual-functional polymers were truly synergistic or merely additive. While polymers containing agmatine and L-arginine were synthesized *via* PPM (section 0) due to their available primary amine groups, thymol and farnesol required direct polymerization through their corresponding acrylate monomers.

A copolymerization of HEAAm with 5 mol% farnesol acrylate and 2 mol% surf-EH3A was successfully performed (e. g. Figure 36). The synthesis yielded a white polymer (**40**), with NMR analysis suggesting about 4 mol% farnesol acrylate and 2-3 mol% surf-EH3A incorporation. GPC analysis revealed an additional shoulder in the molar mass distribution, suggesting secondary reactions potentially involving the double bonds of farnesol during the polymerization process. In contrast, when the same polymerization was repeated using thymol acrylate instead of farnesol acrylate, a colorless polymer was obtained (**41**). GPC analysis showed a single peak in the molar mass distribution, indicating a more straightforward polymerization process without secondary reactions. These differences in polymerization behavior between the two biofilm disruptors highlight the importance of monomer structure in determining the final polymer characteristics. Both polymers were re-made without surf-EH3A.

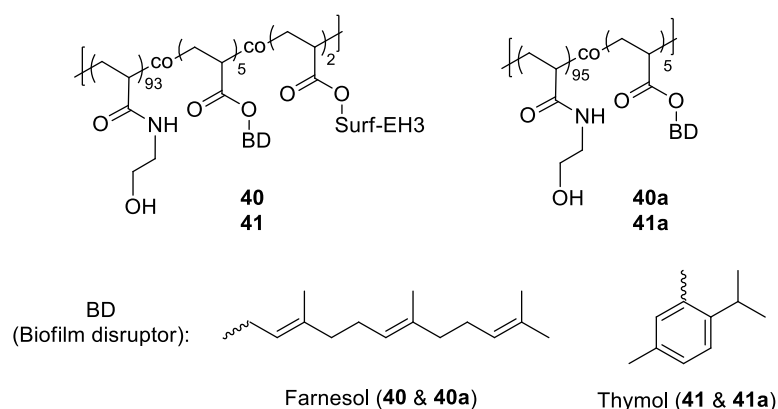


Figure 36: Polymers **40**, **40a**, **41** & **41a** containing biofilm disruptors (thymol or farnesol) without aPDT units.

Summary of the chemical synthesis section

This chapter established two complementary synthetic routes for incorporating photodynamic and biofilm-disrupting agents into polymer systems: post-polymerization modification (PPM) via active ester chemistry and direct free radical polymerization (FRP) of functionalized monomers. Both approaches proved effective for creating water-soluble, functional polymers suitable for antimicrobial applications.

Key synthetic contributions include:

- Development of photosensitizer units (ruthenium complexes and phenalenone derivatives) bearing aliphatic spacers to enable efficient polymer incorporation. The spacer strategy overcame the poor reactivity of aromatic amines in PPM reactions, significantly improving modification efficiency.
- Refinement of ruthenium complex synthesis routes, particularly the one-pot synthesis of compounds **12** and **13**. For compound **12**, this approach eliminated chromatographic purification through efficient precipitation with NH_4PF_6 . For compound **13**, HCl-mediated Boc deprotection provided cleaner products compared to TFA-based methods by generating only volatile byproducts.
- Establishment of efficient synthetic routes to phenalenone monomers, with method optimization improving yields from <5% to >25% for key intermediates and ultimately achieving 65% yield for the final acrylate monomer.
- Incorporation of biofilm disruptors (L-arginine, agmatine, farnesol, thymol) through method-appropriate strategies: PPM for amine-containing compounds and FRP for those requiring monomer derivatization.

These synthetic developments represent the first systematic integration of both photodynamic and biofilm-disrupting functionalities into polymer-based delivery systems for respiratory applications.

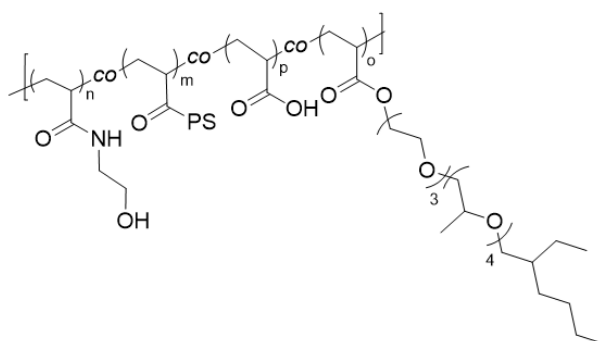
The work produced a diverse library of polymers with varying compositions (aPDT units: 0.5-2 mol%; biofilm disruptors: 0-5 mol%; HEAAm: ~90-97 mol%; surfactant: 2 mol%) and controllable charge characteristics (positive, negative, or neutral). Several polymers were selected for comprehensive biological evaluation based on their distinct structural features and synthetic origins.

3.4 Results from Physiochemical and Biological Evaluations

The biological evaluations presented in this chapter were performed by Dr. Tony Le Gall and his group at the University of Brest, France.

Following the successful synthesis and characterization of the aPDT polymers described in the previous chapter, a comprehensive biological evaluation was conducted to assess their potential as antimicrobial photodynamic therapy agents against a series of bacteria featuring various characteristics like cell wall staining behavior (gram-negative versus gram-positive), respective species, and antibiotic resistance profile. From the library of synthesized polymers, seven key candidates were selected for detailed biological investigation: **26**, **27**, **28**, **33**, **36**, **36a**, and **37**, which are shown in Figure 37 and the detailed composition is described in **Table 1**. These polymers were chosen to represent distinct structural features and synthetic approaches, encompassing both FRP and PPM strategies, as well as varying ratios of photosensitizer units and functional monomers. This systematic variation in composition enables investigation of structure-activity relationships.

Tested polymers:



Photosensitizer (PS):

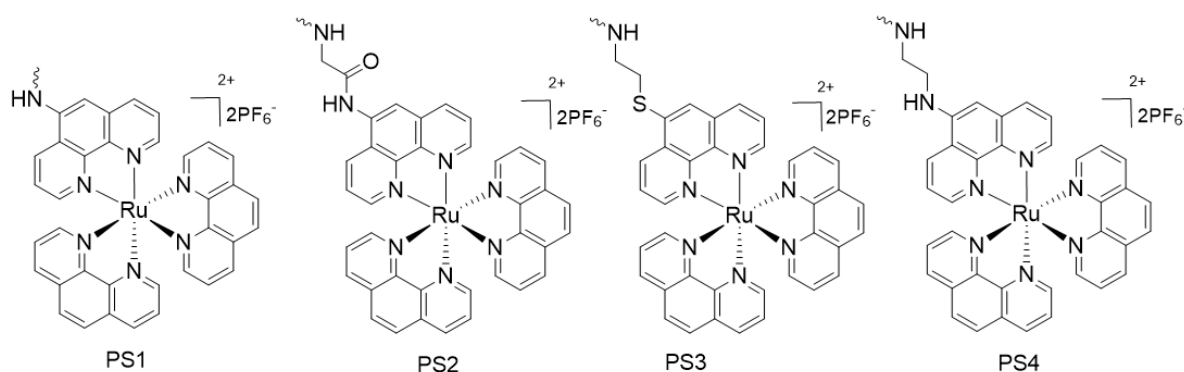


Figure 37: Generic chemical structure of the polymers investigated in this chapter.

Table 1: Detailed composition of the polymers obtained through either of two synthesis strategies, i.e. copolymerization or post-polymerization modification.

aPDT polymers and polymer backbones	m [Ru] Mol%	PS	n HEAAm* Mol%	o Surfactant Mol%	p AA* Mol%	Synthesis strategy
42	0	-	98.0	2.0	0	FRP
43	0	-	98.0	2.0	0	RAFT+PPM
26	1.3	PS4	76.7	2.0	20	PPM
27	0.5	PS3	77.5	2.0	20	PPM
28	2.0	PS2	25.0	2.0	71	PPM
33	1.0	PS3	77.0	2.0	20	FRP
36	0.6	PS1	95.0	4.4	0	FRP
36a	0.5	PS1	99.5	0.0	0	FRP
37	1.0	PS3	97.0	0.0	0	FRP

*Acrylic acid (AA, indicating the negative charge of the final polymer); HEAAm, *N*-hydroxyethyl acrylamide. Polymer **26** contains RAFT chain-end.

While the synthetic work yielded a broader range of polymer candidates, time constraints necessitated focusing on this selection, with additional polymers from the library remaining as promising candidates for future investigations.

The biological assessment followed a systematic approach, beginning with fundamental characterization of charge properties through electrophoresis, followed by evaluation of singlet oxygen production capabilities - the key mechanistic element of photodynamic therapy. The antimicrobial efficacy was then investigated against both planktonic bacteria and biofilms, with particular attention to clinically relevant conditions including varying ionic strengths and increasing the complexity of biological media composition. Importantly, the safety profile of these materials was assessed using human bronchial epithelial cells, and their compatibility with potential therapeutic delivery methods was evaluated through nebulization studies.

First, the optical properties of the synthesized polymers were characterized through UV-visible absorption and phosphorescence emission spectroscopy (Figure 38). The spectra confirm successful incorporation of ruthenium complexes into the polymer systems, with characteristic metal to ligand charge transfer (MLCT) bands around 450

nm in the absorption spectra and emission bands centered around 600 nm in the emission spectra.

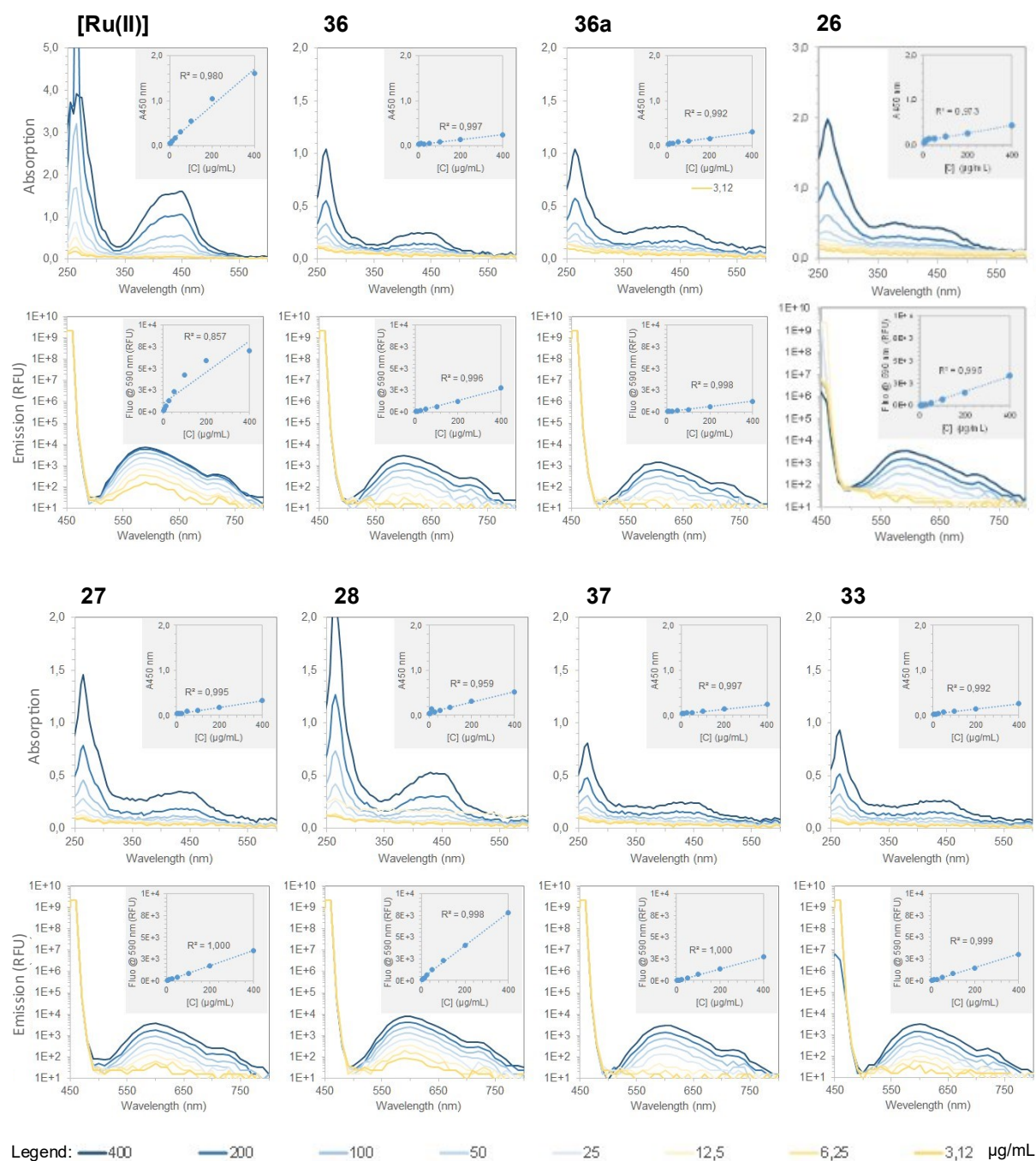


Figure 38: UV-visible absorption spectra (top panels) and phosphorescence emission spectra (following excitation at 450 nm - bottom panels) of [Ru(II)] complex and different polymers at various concentrations (3.12-100 μg/mL). All samples showed linear concentration-dependent increases in absorption and emission intensity.

Polymer **26** exhibited an additional absorption band around 350 nm, which is attributed to the trithiocarbonate chain-end from the RAFT polymerization process used to synthesize its precursor polymer. This feature is absent in other polymers that were

synthesized from FRP-polymerized precursors (**27**, **28**) or via direct FRP (**36**, **36a**, **37**, **33**).

Both absorption and emission intensities increased linearly with concentration for all samples (3.12-100 $\mu\text{g/mL}$). Polymer **28** showed higher phosphorescence intensity at equivalent concentrations compared to other polymers, consistent with its higher ruthenium complex content (2 mol% vs. 0.5-1 mol% in other polymers).

3.4.1 Electrophoresis Test

The electrophoretic mobility of antimicrobial photodynamic polymers was investigated to understand their charge characteristics, a crucial factor determining bacterial cell membrane interactions and therapeutic efficacy. Gel electrophoresis, which separates molecules based on their charge-to-mass ratio in an electric field, revealed distinct charge patterns, as shown in Figure 39 that correlated with the synthesis method employed.

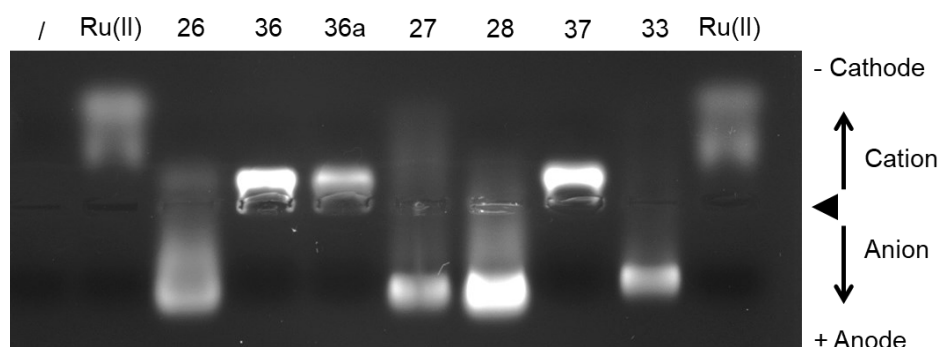


Figure 39: Agarose gel electrophoresis of [Ru(II)] and the various PDT polymers. The black triangle indicates the position of the wells (where samples were deposited; 2 μ g of [Ru(II)] or 20 μ g of aPDT polymer, respectively) and the vertical arrows denote the direction of migration of reagents during electrophoresis, depending on whether they exhibit a net cationic or anionic charge.

Polymers synthesized *via* PPM exhibited predominantly negative charge characteristics (**26**, **27**, **28**), an unexpected outcome attributed to the partial hydrolysis of active ester units to free carboxylic acid groups during the modification process. A similar negative charge was achieved intentionally in **33** through free radical polymerization (FRP) by incorporating acrylic acid as a comonomer, demonstrating that desired charge characteristics could be obtained through different synthetic approaches. In contrast, polymers prepared through free radical copolymerization (**36**, **36a**, **37**) showed positive charges, primarily due to the incorporation of the cationic ruthenium complex. The phosphorescence of the Ru(II) complexes enabled direct visualization of the PS-modified polymers under UV irradiation (340 nm) without additional staining, while control polymers lacking the Ru(II) complex showed no detectable signal. This observation confirmed successful conjugation of the photosensitizer units to the polymer backbone (Figure 40).

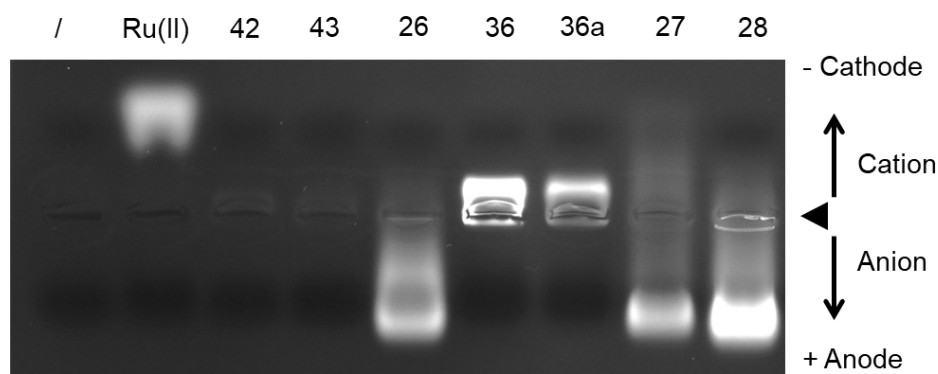


Figure 40: Agarose gel electrophoresis of [Ru(II)], polymer backbones devoid of Ru moieties and various PDT polymers. The black triangle indicates the position of the wells (where samples were deposited; 2 μ g of [Ru(II)] or 20 μ g of polymer, respectively) and the vertical arrows denote the direction of migration of reagents during electrophoresis, depending on whether they exhibit a net positive or negative charge.

These observations led to modifications in polymer design: while the negative charge in PPM polymers resulted from an unintended hydrolysis, the successful incorporation of acrylic acid through FRP (as demonstrated in **33**) provided a controlled method to introduce negative charges. The success of this strategy was confirmed by electrophoretic analysis, where polymers with varying acrylic acid content showed predictable migration patterns toward the positive pole, indicating the dominance of negative charges over the ruthenium complex's positive contribution.

The electrophoretic behaviour was also examined under two distinct conditions to provide more comprehensive insights, as shown in Figure 41. First, polymers were analysed in water to establish their inherent charge characteristics. Second, the polymers were studied in a diluted artificial sputum medium (ASM), a complex, mucus-mimicking environment containing various biomacromolecules, including the polyanion DNA.[138]

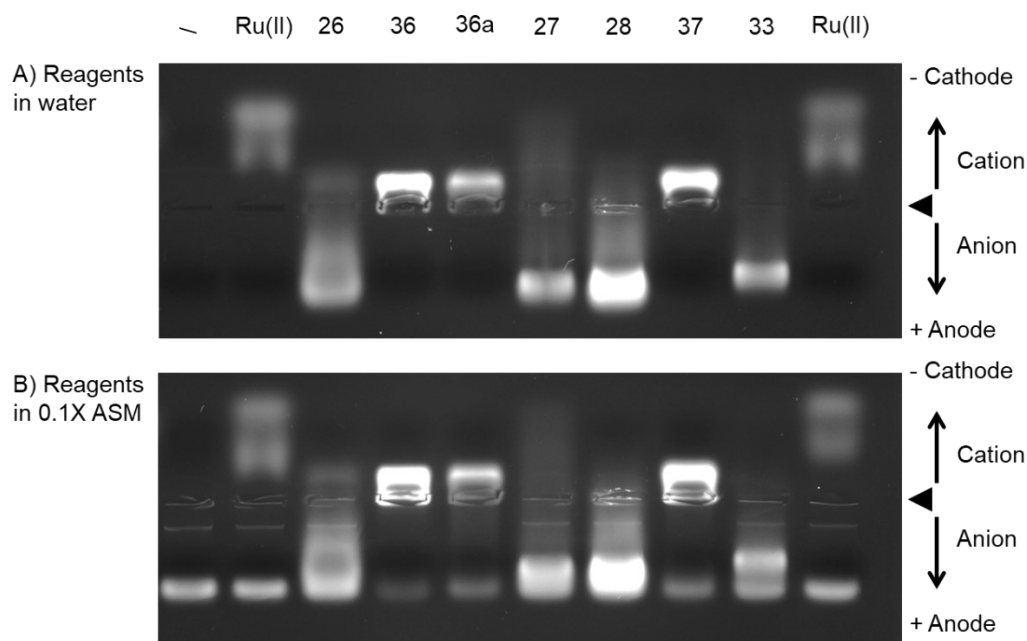


Figure 41: Agarose gel electrophoresis of [Ru(II)] and the various aPDT polymers: comparison of reagents in water (A) or mixed with an artificial sputum medium (ASM, B). The black triangle indicates the position of the wells (where samples were deposited; 2 μ g of [Ru(II)] or 20 μ g of aPDT polymers, respectively) and the vertical arrows denote the direction of migration of reagents during electrophoresis, depending on whether they exhibit a net positive or negative charge, respectively. The bottom band corresponds to DNA (that is contained in ASM), which observation was possible due to the staining of the gel with ethidium bromide (EtB) (at 0.5 μ g/mL).

The DNA content in ASM, visualized using ethidium bromide (EtB) staining, served as an internal marker and revealed interesting polymer-biomolecule interactions. Positively charged polymers (**36**, **36a** & **37**) demonstrated notable DNA complexation, evidenced by band retardation and intensity changes in the DNA signal. This interaction was specific to the polymeric materials, as free ruthenium complex, despite its positive charge, showed no DNA binding capability, highlighting the importance of the polymer backbone in facilitating these interactions.

The consistency of results across different solvents (water or saline) and the observation of single, discrete bands with migration patterns distinct from free ruthenium complex confirmed both the successful polymer conjugation and the robust nature of their charge characteristics. These findings not only validated the synthetic strategies employed but also provided valuable insights into the potential behaviour of these materials in biological environments, particularly their ability to interact with charged cellular components, which is crucial for their intended antimicrobial applications. The evaluation proceeded to examine singlet oxygen production

capabilities, one key mechanistic element underlying photodynamic therapy efficacy.[51]

3.4.2 Singlet Oxygen Sensor Green Assay

The production of singlet oxygen ($^1\text{O}_2$), one primary reactive oxygen species in photodynamic therapy, was quantified using singlet oxygen sensor green (SOSG) reagent. This specific fluorogenic probe exhibits enhanced fluorescence upon reaction with $^1\text{O}_2$ through an electron transfer (ET)-controlled mechanism, forming SOSG endoperoxide (SOSG-EP), as detailed in Figure 42. The resulting fluorescence intensity directly correlates with singlet oxygen production, enabling quantitative assessment of photodynamic efficiency.

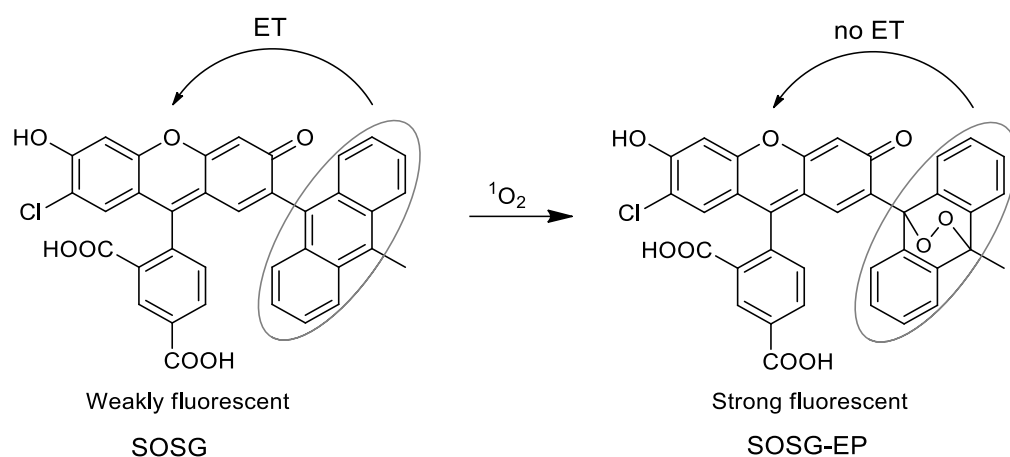


Figure 42: SOSG mechanism showing the conversion of weakly fluorescent SOSG to strongly fluorescent SOSG-EP through reaction with singlet oxygen.

Initial screening of singlet oxygen production was conducted across a range of concentrations (up to 500 $\mu\text{g/mL}$) for both [Ru(II)] complex and aPDT polymers in water, which is shown in Figure 43. The concentration-dependent studies revealed varying profiles among the tested materials, with some polymers exhibiting a bell-shaped response curve, likely due to saturation or quenching effects at higher concentrations. To enable direct comparison, subsequent analyses were performed at 50 $\mu\text{g/mL}$, a concentration that provided linear response for all materials tested.

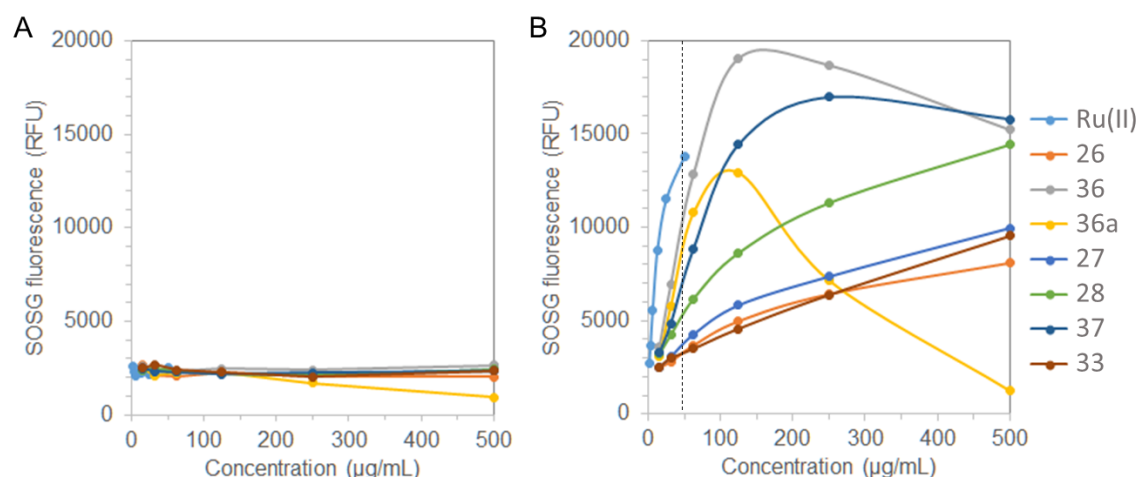


Figure 43: Detection of singlet oxygen production of [Ru(II)] and the various PDT polymers: fluorescence of the SOSG dye before (A) and after (B) exposure to light. Reagents were tested for concentrations up to 500 µg/mL in water. The dashed vertical line in (B) specifies the concentration 50 µg/mL up to which a linear dose/response is obtained with all reagents. The data correspond to $n=1$. RFU, relative fluorescence unit.

Comparative analysis in water revealed that all materials could generate singlet oxygen upon specific light irradiation at 450 nm, though with varying efficiencies, details are demonstrated in Figure 44.

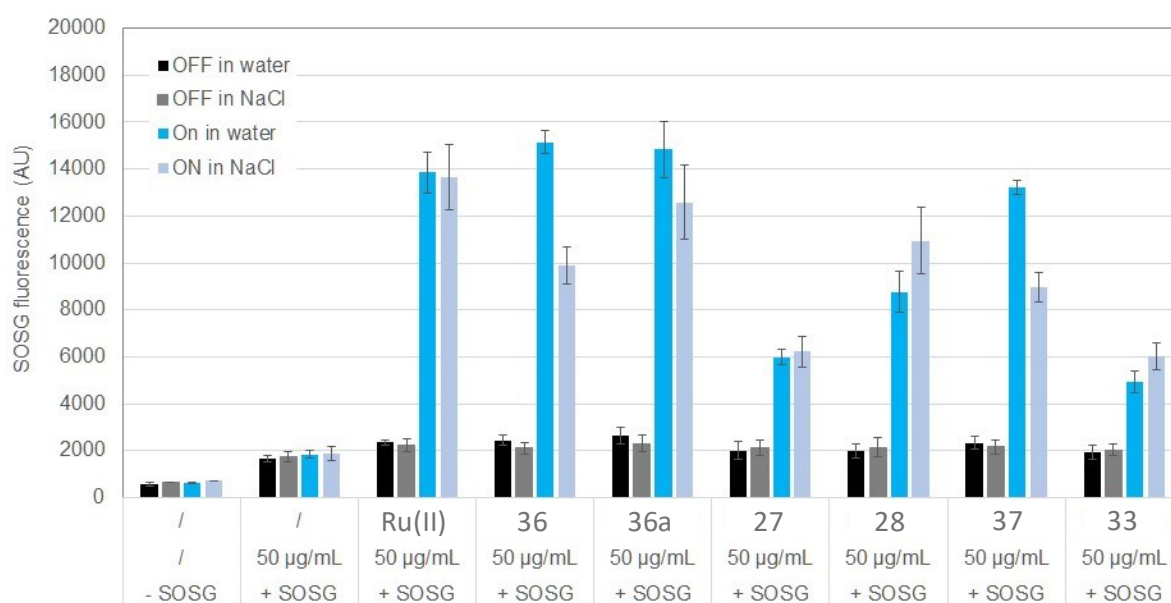


Figure 44: Detection of singlet oxygen production of [Ru(II)] and the various PDT polymers: comparison in water or 1X NaCl. The fluorescence of the SOSG dye was measured before (OFF) and after (ON) exposure to light. All reagents were tested at 50 µg/mL. Measurements were done after three successive 5-min irradiations (cumulated irradiations = 15 min). The data correspond to mean $\pm$ SD with $n=3$. RFU, relative fluorescence unit.

The aPDT polymers demonstrated characteristic differences in their $^1\text{O}_2$ production capability: **36** and **36a** showed efficiency comparable to free [Ru(II)], followed by **37**, **28**, while **27** and **33** exhibited the lowest production. Interestingly, no direct correlation

was observed between the ruthenium content and $^1\text{O}_2$ generation efficiency. For instance, **28**, despite having the highest Ru content, showed moderate production, while **36** and **36a**, with lower Ru content, were among the most efficient producers. These differences might be attributed to various factors including the type of Ru complex used (PS1-4), the net charge of the polymers (particularly relevant for negatively charged **27**, **28**, and **33**), or specific aspects of the polymer architecture.

Additionally, the interpretation of singlet oxygen detection using SOSG should consider several important factors. The negative charge of SOSG can influence its interaction with the polymers depending on their charge characteristics, potentially affecting the detection efficiency. Additionally, while singlet oxygen is a highly reactive species, its very short lifetime and limited diffusion distance mean that detection requires SOSG to be physically close to where singlet oxygen is generated. Furthermore, differences in oxygen distribution between the aqueous medium and the more hydrophobic polymer microenvironment may affect singlet oxygen production, as polymer coils can concentrate oxygen locally, potentially enhancing $^1\text{O}_2$ generation within the polymer domain. Therefore, the observed singlet oxygen production rates may be influenced by both the actual generation efficiency and how close SOSG can get to the photosensitizer.

The influence of ionic strength was investigated by comparing $^1\text{O}_2$ production in water versus saline conditions (1x NaCl). While the free [Ru(II)] complex showed consistent performance across both conditions, the PDT polymers exhibited varying sensitivities to the ionic environment. **36** and **37** showed marked sensitivity to salt presence, whereas **27** and **33** remained largely unaffected. Notably, **28** demonstrated enhanced $^1\text{O}_2$ production in saline conditions, highlighting how medium composition can significantly impact the photodynamic efficiency of these materials.

Kinetic studies of $^1\text{O}_2$ production, monitored through SOSG fluorescence over multiple irradiation cycles, provided additional insights into the performance characteristics of these materials, as shown in Figure 45.

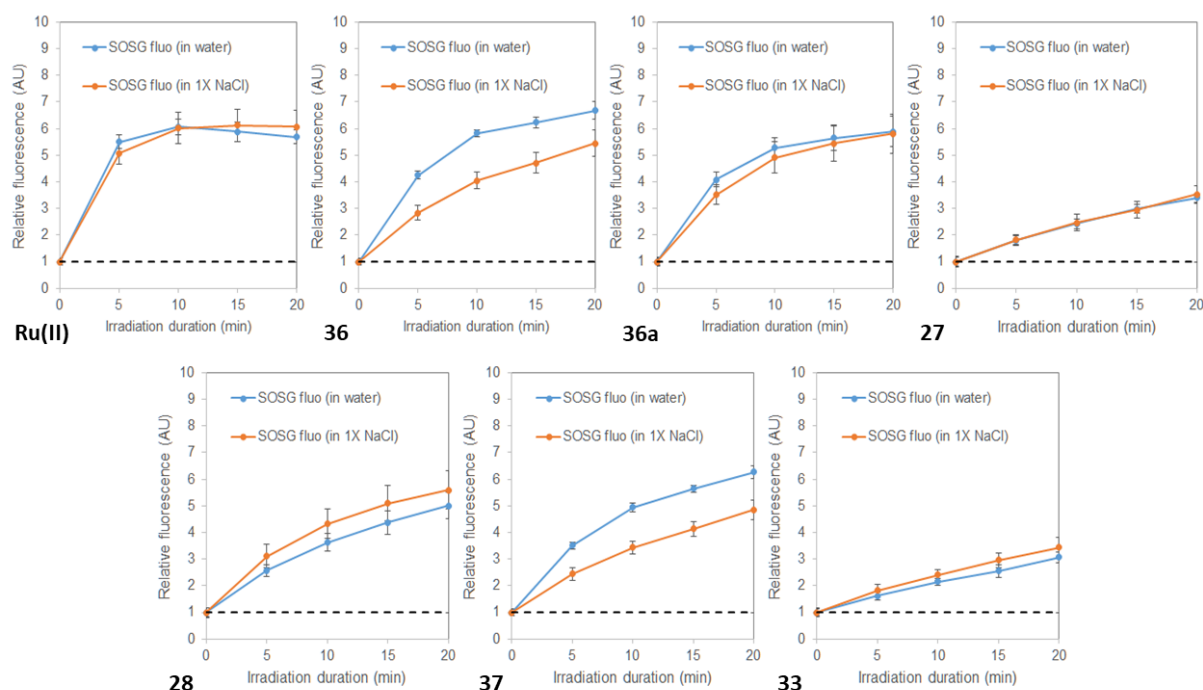


Figure 45: Kinetic of singlet oxygen production of [Ru(II)] and the various PDT polymers: effect of the irradiation duration for tests done either in water or in 1x NaCl. Reagents were tested at 50 $\mu\text{g/mL}$. Measurements were done after four successive 5-min irradiations. The dashed line refers to signal measured before light irradiation. The data correspond to mean $\pm$ SD with $n=3$.

The free [Ru(II)] complex reached maximum production after the first irradiation cycle, maintaining a plateau thereafter. In contrast, PDT polymers showed gradual increases in $^1\text{O}_2$ production over successive irradiations, with distinct kinetic profiles reflecting their composition. Two distinct kinetic patterns emerged: **36**, **36a**, **28**, and **37** showed rapid initial increases followed by plateaus, while **27** and **33** exhibited linear increases in singlet oxygen production over time. All materials eventually approached similar maximum levels, suggesting complete consumption of the SOSG dye. Notably, despite their different synthetic routes, the two polymers **27** and **33** demonstrated remarkably similar properties across all experimental conditions tested.

Photochemical stability studies

Beyond singlet oxygen production capacity, the photochemical stability and recovery characteristics of these materials under extended irradiation were investigated. Studies of emission behavior included both photofading during light exposure and potential recovery during subsequent dark storage (Figure 46).

In the absence of light, all compounds maintained relatively stable emission, showing only minor decreases (10-20%) over 60 minutes. However, under continuous light

exposure, significant differences emerged. **26** showed the most pronounced photochemical response, with emission decreasing by approximately 80% after 50 minutes of irradiation. This enhanced photofading in polymer **26** may involve the trithiocarbonate RAFT chain-end, which could undergo photodegradation under the irradiation conditions, potentially affecting the polymer's overall photostability.[139] **36a** exhibited moderate photofading (about 40% decrease), while **27** and **28** demonstrated superior photostability with only 20-30% reduction in emission intensity.

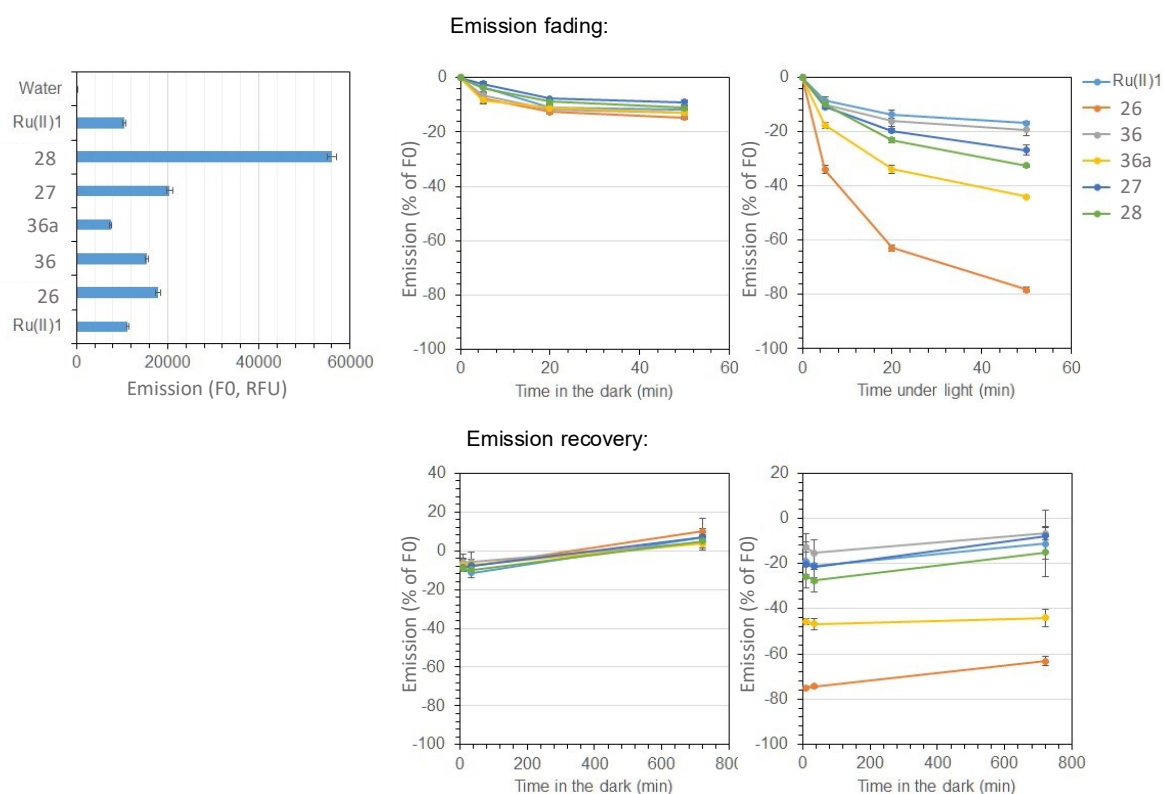


Figure 46: Emission studies of Ru(II) and aPDT polymers. Top panel shows emission fading: Initial emission intensity in water (left), emission stability in dark conditions (middle), and photofading under light exposure (right). Bottom panel shows emission recovery experiments: Monitoring of emission intensity during prolonged dark storage (800 min) after light-induced fading, demonstrating incomplete recovery of the original emission intensity. Ru(II)1 and PDT polymers were tested in water at 12.5 and 125 $\mu\text{g/mL}$, respectively.

Subsequent monitoring of Emission recovery in dark conditions revealed incomplete restoration of the original Emission intensity, even after prolonged dark storage (up to 800 minutes). This incomplete recovery suggests that some photochemical changes may be irreversible, though UV-visible absorption spectra remained unchanged across all materials regardless of light exposure, indicating preservation of the basic molecular structure.

3.4.3 Assays using Planktonic Bacterial Cells

Interaction tests in water or in saline

The characterization of aPDT polymer-bacteria interactions was conducted using several bacterial strains with distinct characteristics (Table 2). This selection included both Gram-negative (*P. aeruginosa*, *E. coli*) and Gram-positive (*S. aureus*) bacteria, enabling evaluation of the polymers against organisms with different cell wall structures, antibiotic resistance profiles, and clinical relevance. These strains were selected also with respect to their relevance with CF lung infections. In most assays below, at least one strain from each species was included.

Table 2: Bacteria used in this study.

Species	Strain	Origin and other features
<i>Staphylococcus aureus</i> (SA)	Newman[140]	Clinical (human infection), MDS
	N315[141]	Clinical (nosocomial), MRSA
<i>Escherichia coli</i> (EC)	DH5alpha	Laboratory, MDS
<i>Pseudomonas aeruginosa</i> (PA)	PA19660[72]	Laboratory, MDS, mucoid
	CH2678*	Clinical (sputum CF), MDR
	F1758*	Clinical (tracheobronchial secretion), MDS

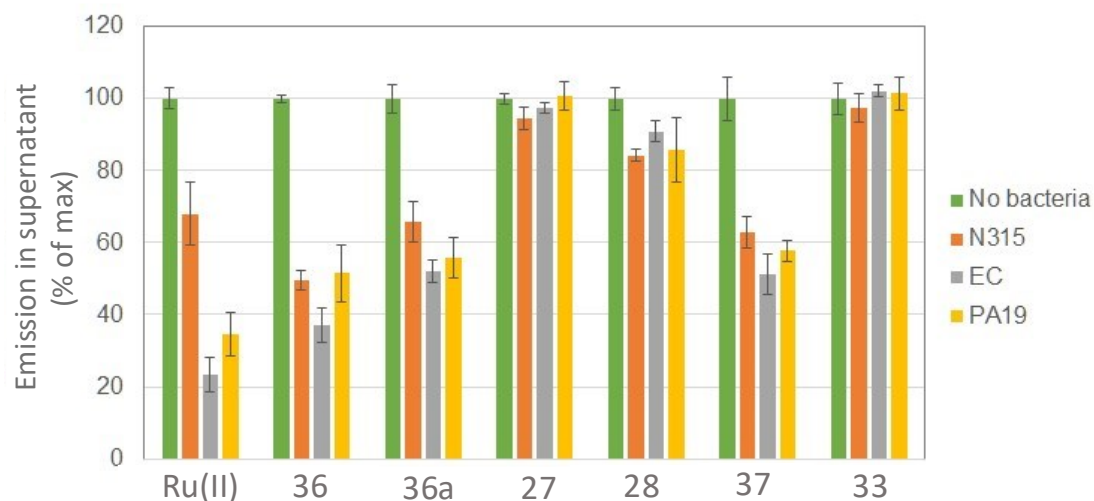
MRSA, methicillin resistant *S. aureus*; MDS, “multidrug susceptibility” (susceptibility to most antimicrobial agents); MDR, “multidrug-resistance” (resistance to at least one agent in three or more antimicrobial categories). CF, cystic fibrosis; mucoid, production of gelatinous or viscous extracellular polymeric substances (EPS). *Bactome collection.[142]

The inherent luminescent properties of the ruthenium complexes enabled quantitative monitoring of polymer-bacteria interactions. A simple yet effective centrifugation-based separation method was employed to evaluate these interactions under different conditions, see Figure 47. In this assay, bacteria were incubated at 37°C for 30 min with aPDT polymers, followed by centrifugation to separate the bacterial cells (pellet) from the surrounding medium (supernatant). While this method provides a straightforward assessment of polymer-bacteria association through supernatant emission depletion, direct emission measurement of bacterial pellets could provide additional sensitivity for detecting weaker interactions.

The emission intensity of the supernatant was then measured, with any decrease in emission compared to the control (no bacteria) indicating polymer association with the

bacterial cells. This methodology was applied across the three different bacterial species (PA19, N315 and EC) under two distinct conditions: water and physiological saline (1X NaCl).

A) Test in water



B) Test in 1X NaCl

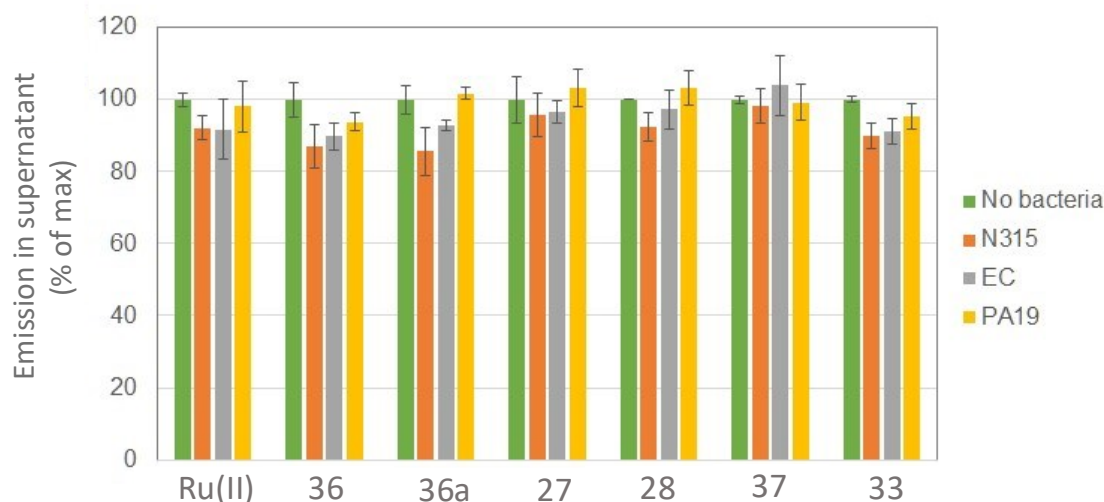


Figure 47: Interaction of [Ru(II)] and the various aPDT polymers: evaluation of three bacterial species. Tests were done when mixing bacteria with the reagents (at 5 or 50 $\mu\text{g/mL}$ for Ru(II) and aPDT polymers, respectively) either in water (A) or in 1X NaCl (B). The data correspond to mean $\pm$ SD with $n=3$.

In water, clear charge-dependent interaction patterns emerged. The positively charged materials - [Ru(II)] complex and aPDT polymers **36**, **36a**, and **37** - showed significant decreases in supernatant emission across all bacterial species tested, indicating substantial bacterial association. This interaction can be attributed to electrostatic attraction between the positively charged polymers and the naturally negative surface charge of bacterial cell membranes. In contrast, the negatively charged aPDT

polymers (**28**, **27**, and **33**) exhibited minimal to no reduction in supernatant emission, suggesting limited or weak bacterial interaction, likely due to electrostatic repulsion.

When the same experiments were conducted in saline conditions (1X NaCl), a dramatic change in interaction patterns was observed. The presence of salt effectively neutralized all polymer-bacteria interactions, as evidenced by negligible changes in supernatant emission regardless of the polymer's charge characteristics or bacterial species. This phenomenon can be explained by the ionic screening effect of Na⁺ and Cl⁻ ions, which shield the electrostatic charges and prevent the charge-based interactions observed in water. This behavior parallels the previously observed DNA interaction patterns, highlighting the crucial role of electrostatic forces in mediating polymer-bacteria associations.

The results demonstrate that the ability of aPDT polymers to interact with bacterial cells is primarily governed by electrostatic interactions, which can be modulated by environmental conditions such as ionic strength. This understanding is crucial for optimizing the design and application of these materials in different physiological contexts.

Minimal inhibitory concentration (MIC) determination

The aPDT efficacy of [Ru(II)] and aPDT polymers was evaluated through minimal inhibitory concentration (MIC) determinations against three bacterial species: *P. aeruginosa* (PA19), *S. aureus* (N315), and *E. coli* (EC).

The experiments were conducted both in dark conditions and under blue light irradiation for 30 minutes, details are shown in **Table 3**.

Table 3: MIC (in $\mu\text{g/mL}$) of [Ru(II)] and PDT polymers: determination using three bacterial species.

	PA19		SA N315		EC	
	OFF	ON	OFF	ON	OFF	ON
DMSO	>1%	>1%	>1%	>1%	>1%	>1%
[Ru(II)]	>50	0.8	>50	3.1	>50	3.1
36	>500	7.8	>500	15.6	>500	3.9
36a	>500	15.6	>500	31.3	>500	15.6
27	>500	0.5	>500	>500	>500	>500
28	>500	>500	>500	>500	>500	>500
37	>500	15.6	>500	31.3	>500	15.6
33	>500	62.5	>500	>500	>500	>500

Measurements were done when mixing bacteria and reagents in water and under dark condition (OFF) or after exposure to light for 30 min (ON). Reagents were tested for concentrations ranging from 0.05 to 50 $\mu\text{g/mL}$ (Ru) or from 0.5 to 500 $\mu\text{g/mL}$ (aPDT polymers). The bacteria tested were *P. aeruginosa* (PA19), *S. aureus* (N315) and *E. coli* (EC).

The absence of dark toxicity across all materials tested, even at the highest concentrations (500 $\mu\text{g/mL}$ for aPDT polymers and 50 $\mu\text{g/mL}$ for [Ru(II)]), demonstrated the strict light-dependence of their antimicrobial activity. This selective activation represents a key advantage for controlled therapeutic applications.

Upon blue light irradiation, distinct patterns of antimicrobial activity emerged, revealing both material-dependent and species-dependent effects:

Material-dependent effects:

- [Ru(II)] showed consistent effectiveness across all bacterial species.
- **36**, **36a**, and **37** demonstrated strong antimicrobial activity for all species.
- **28** showed no significant antimicrobial effect under any condition.

- **27** and **33** exhibited species-dependent efficacy specifically for *P. aeruginosa*.

Species-dependent responses:

- *P. aeruginosa* showed sensitivity to all active materials.
- *S. aureus* demonstrated selective sensitivity, responding only to [Ru(II)], **36**, **36a**, and **37**.
- *E. coli* displayed similar sensitivity patterns to *S. aureus*.

To further understand the photodynamic response, detailed investigations of irradiation protocols were conducted. The experimental design, as shown in Figure 48, employed both continuous and discontinued irradiation protocols, with bacterial viability monitored through spot plating on LB agar. This methodology provided clear visualization of the antimicrobial effects and their dependence on both concentration and light exposure parameters.

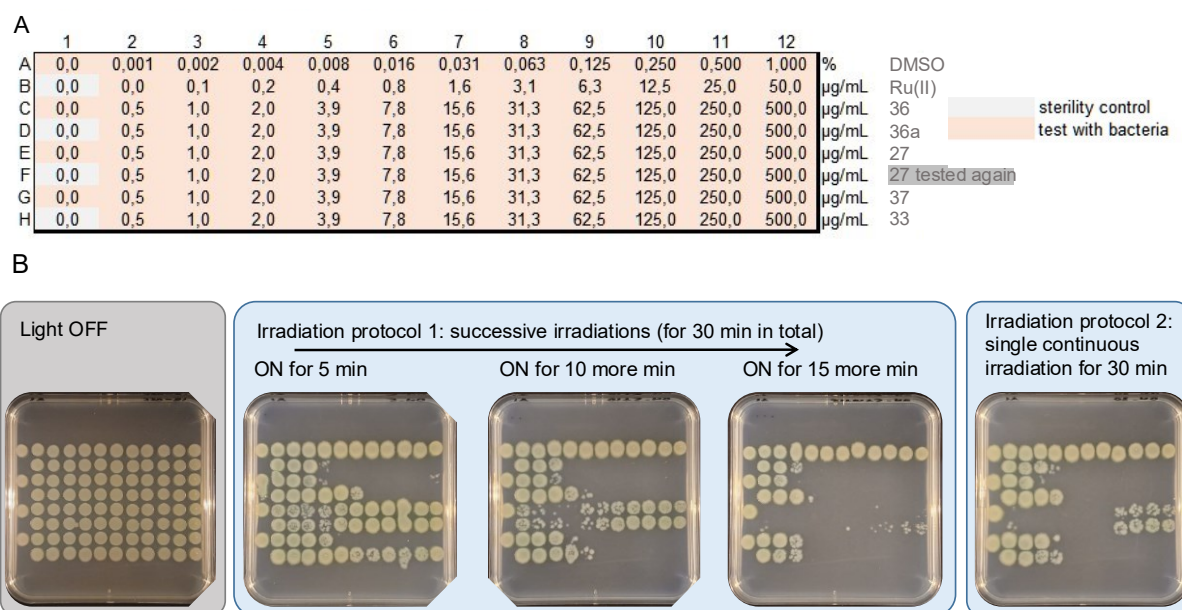


Figure 48: aPDT of [Ru(II)] and PDT polymers towards planktonic cells of *P. aeruginosa* PA19: effects of light duration and irradiation protocols. (A) Layout of the experiment and (B) bacterial growth before and after exposure to light following a discontinued or continuous irradiation protocol. [Ru(II)] and PDT polymers were tested in water for concentrations ranging from 0.1 to 50 µg/mL and 0.5 to 500 µg/mL, respectively. At the time points indicated, 5 µL from every sample was spotted on LB agar. After incubation at 37°C for several hours, the presence of growing bacteria allowed to determine whether bacteria survived the applied treatment.

These studies revealed important differences in the kinetics of antimicrobial activity among the aPDT polymers:

- Fast-acting materials: **36**, **36a**, and **37** achieved maximum effect without requiring extended irradiation times.
- Slow-acting materials: **27** and **33** showed enhanced antimicrobial activity with increased irradiation duration.

This variation in response to irradiation time correlates with the previously observed singlet oxygen production kinetics (see Figure 45), where **27** and **33** demonstrated slower rates of $^1\text{O}_2$ generation. Additionally, the extended irradiation protocols resulted in longer polymer-bacteria contact times, which may also contribute to the enhanced antimicrobial effects observed with increased irradiation duration. Of particular interest was polymer **27**'s concentration-dependent behavior, which exhibited higher aPDT activity at intermediate concentrations, while higher concentrations showed reduced antimicrobial efficacy. This unusual dose-response pattern was reproducible and may indicate concentration-dependent aggregation or self-quenching effects that warrant further investigation.

Determination of bacterial load reduction

The antimicrobial efficacy of [Ru(II)] and aPDT polymers was quantitatively assessed by determining bacterial load reduction across multiple bacterial species in both water and saline conditions, see Figure 49. This analysis provided deeper insights into the antimicrobial activity previously observed in MIC determinations, while also revealing species-specific responses to photodynamic treatment.

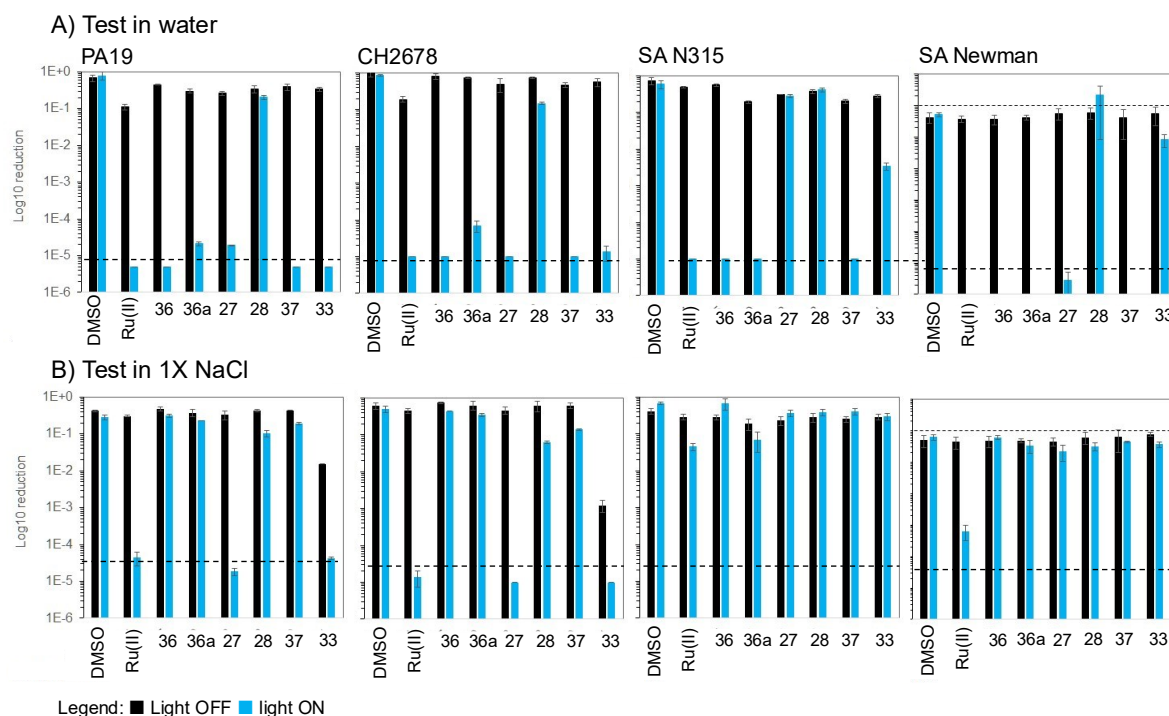


Figure 49: Logarithmic (Log10) reduction of colony-forming units (CFU) in planktonic bacteria treated with [Ru(II)] or aPDT polymers either kept in the dark (OFF) or exposed to light (ON): comparison of the results obtained for tests done in water (A) or in 1X NaCl (B). The bacteria tested were Gram negative *P. aeruginosa* (PA19 as a standard strain and CH2678 as a clinical strain) and Gram-positive *S. aureus* (MRSA N315 and Newman). The data correspond to mean $\pm$ SD with $n=3$. The bottom dashed line refers to the limit of detection.

In aqueous conditions, the Gram-negative bacteria *P. aeruginosa* (strains PA19 standard and CH2678 clinical)[72,143] demonstrated significant susceptibility to photodynamic treatment. All active materials achieved substantial bacterial load reduction upon light activation, with most treatments reaching the detection limit, indicating complete bacterial elimination. Notably, **27** and **33** maintained their effectiveness despite their previously observed slower kinetics of singlet oxygen production, suggesting that even delayed photodynamic responses can achieve complete bacterial elimination given sufficient time.

The Gram-positive bacteria *S. aureus* (strains N315 and Newman)[141,144] exhibited a different sensitivity profile in water. These strains showed high susceptibility to [Ru(II)], **36**, **36a**, and **37**, but demonstrated resistance to **27** and **33**. This selective response pattern aligned perfectly with the MIC determination results and likely reflects the fundamental differences in cell wall structure between Gram-positive and Gram-negative bacteria, as well as the influence of polymer charge on bacterial interaction.

The introduction of physiological saline (1x NaCl) markedly altered the antimicrobial efficacy patterns. *S. aureus* strains showed substantially reduced susceptibility to all materials under saline conditions, which can be attributed to the ionic screening effect previously observed in interaction studies. However, an interesting phenomenon emerged with *P. aeruginosa* strains, which maintained their sensitivity to **27** and **33** even in saline conditions. This preserved activity suggests these polymers possess unique properties that enable them to overcome ionic interference, possibly through mechanisms independent of purely electrostatic interactions.

These findings were validated through complementary plating experiments (Figure 50), where bacterial survival was systematically evaluated across all strains. In water under light activation, Gram-negative bacteria (PA19, CH2678, EC = *E. coli*) showed elimination with polymers **36**, **36a**, **37**, **27**, and **33**, while Gram-positive bacteria (N315, Newman) were eliminated only by polymers **36**, **36a**, and **37**, showing resistance to **27** and **33**.

In 1× NaCl conditions, antimicrobial activity was substantially reduced for most polymers against all strains. However, polymers **27** and **33** maintained activity specifically against Gram-negative bacteria (PA19, CH2678, EC) even under these screening conditions, while showing no activity against Gram-positive bacteria (N315, Newman). The plate assay results not only corroborated the quantitative load reduction data but also provided visual confirmation of the species-specific and condition-dependent antimicrobial efficacy patterns.

This visual validation proved particularly valuable for understanding the heterogeneity of antimicrobial responses, especially in cases where complete bacterial elimination was not achieved. The observation of spatial control over bacterial survival led to further investigations of precise photodynamic targeting capabilities, as demonstrated in subsequent experiments using masked irradiation patterns. These findings emphasize the potential for controlled, localized antimicrobial treatment using these photodynamic systems.

Layout

 Illumination :
 Light Off
 Light On

	Test in water			Test in 1X NaCl			Test in water			Test in 1X NaCl		
	1	2	3	4	5	6	7	8	9	10	11	12
A	#1	#1	#1	#9	#9	#9	#1	#1	#1	#9	#9	#9
B	#2	#2	#2	#10	#10	#10	#2	#2	#2	#10	#10	#10
C	#3	#3	#3	#11	#11	#11	#3	#3	#3	#11	#11	#11
D	#4	#4	#4	#12	#12	#12	#4	#4	#4	#12	#12	#12
E	#5	#5	#5	#13	#13	#13	#5	#5	#5	#13	#13	#13
F	#6	#6	#6	#14	#14	#14	#6	#6	#6	#14	#14	#14
G	#7	#7	#7	#15	#15	#15	#7	#7	#7	#15	#15	#15
H	#8	#8	#8	#16	#16	#16	#8	#8	#8	#16	#16	#16

#1 & 9: DMSO (cf 1%)
 #2 & 10: Ru(II) (cf 50 µg/mL)
 #3 & 11: 36 (cf 500 µg/mL)
 #4 & 12: 36a (cf 500 µg/mL)
 #5 & 13: 27 (cf 500 µg/mL)
 #6 & 14: 28 (cf 500 µg/mL)
 #7 & 15: 37 (cf 500 µg/mL)
 #8 & 16: 33 (cf 500 µg/mL)

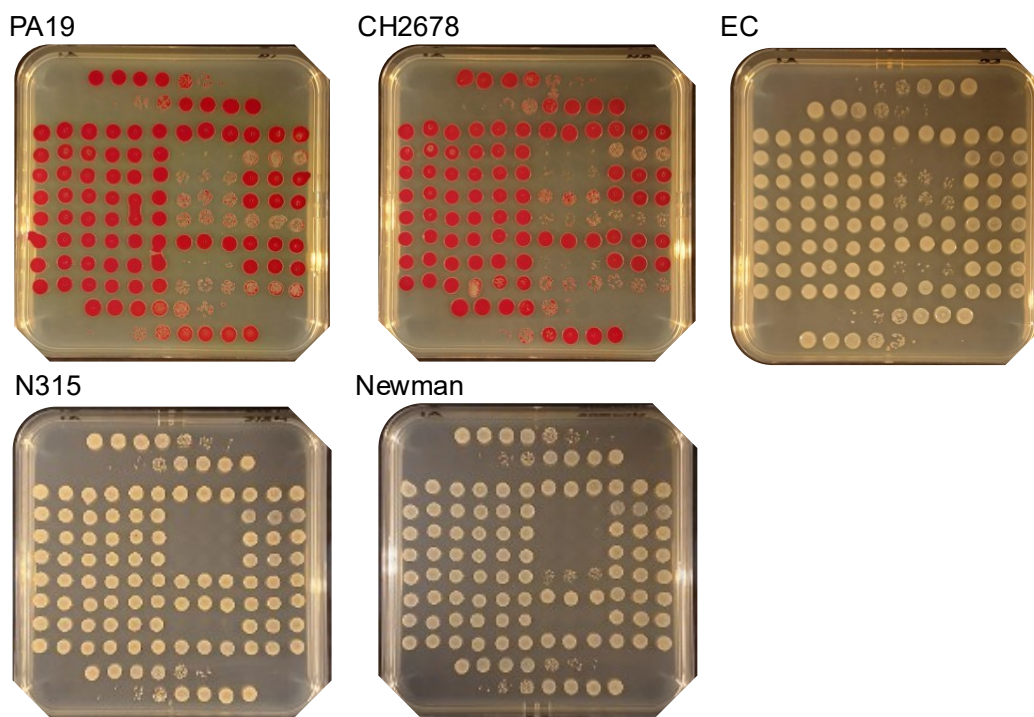


Figure 50: Plate assay showing bacterial survival after treatment with [Ru(II)] and aPDT polymers. Five bacterial strains were tested on separate plates: PA19 and CH2678 (*P. aeruginosa*), EC (*E. coli*), N315 and Newman (*S. aureus*). The layout (top) shows the experimental design applied to all plates: samples were tested in water (left half) or 1× NaCl (right half) under dark conditions (grey, "Light Off") or after light exposure (blue, "Light On"). Sample identities: #1 & 9: DMSO (cf 1%); #2 & 10: [Ru(II)] (cf 50 µg/mL); #3 & 11: 36 (cf 500 µg/mL); #4 & 12: 36a (cf 500 µg/mL); #5 & 13: 27 (cf 500 µg/mL); #6 & 14: 28 (cf 500 µg/mL); #7 & 15: 37 (cf 500 µg/mL); #8 & 16: 33 (cf 500 µg/mL). Red spots indicate bacterial growth; absence indicates elimination. Note: The two top and two bottom rows correspond to 10-fold serial dilutions of the bacterial inoculum.

The spatial control capabilities of photodynamic therapy using [Ru(II)]1 were demonstrated against *P. aeruginosa* strain F1758, as demonstrated in Figure 51. A mask displaying "TARGET THERAPY" with defined transparency gradients (100%, 55%, 30%, 17%, 10%, and 0% light transmission) was used to modulate light exposure across the bacterial culture. Following irradiation and subsequent incubation at 37°C for 16 hours, the bacterial growth pattern precisely reproduced the mask design, with bacterial survival correlating inversely with light transmission intensity.

The high-resolution reproduction of the logo demonstrated the exceptional spatial precision achievable with this photodynamic system, establishing the potential for precisely targeted antimicrobial treatments in complex biological environments.

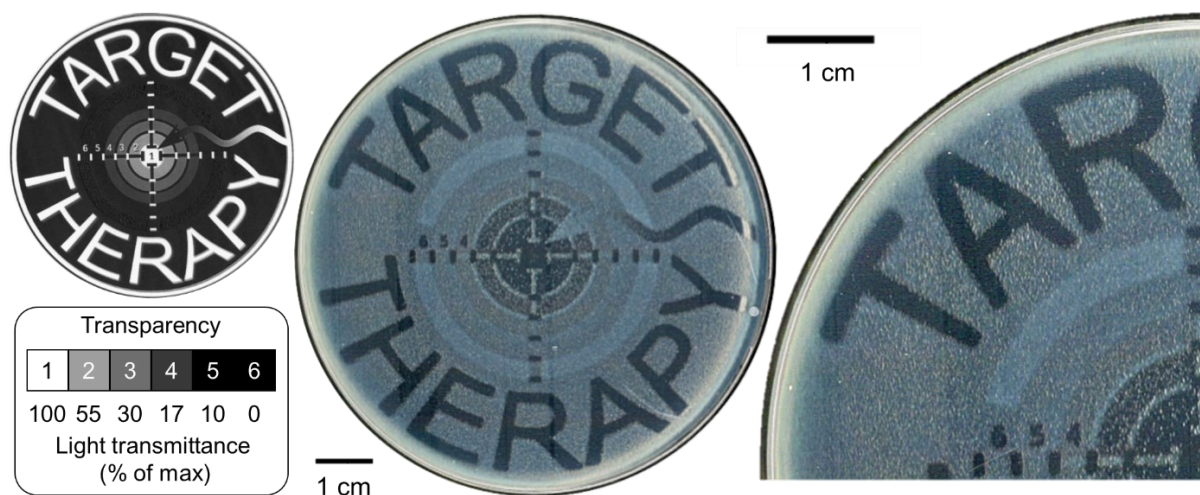


Figure 51: Spatially controlled photodynamic inactivation of planktonic cells of the *P. aeruginosa* clinical strain F1758. The same outcome was observed using the aPDT polymers.

Test at higher salinities and in more complex media

The impact of environmental conditions on antimicrobial photodynamic therapy was further investigated by examining the effects of varying ionic strength and complex biological media on aPDT efficacy. These studies provided crucial insights into the behaviour of [Ru(II)] and the aPDT polymer **26** under more physiologically relevant conditions (Figure 52).

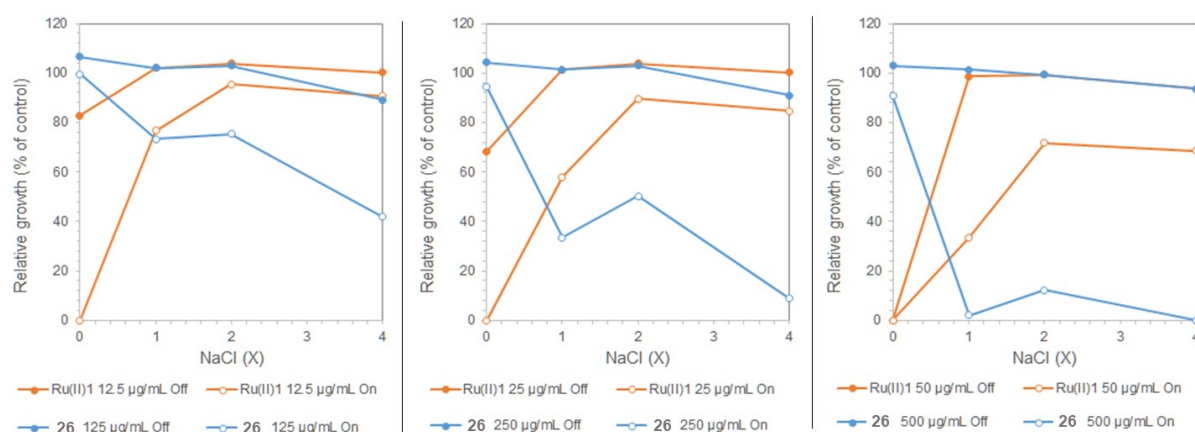


Figure 52: Impact of the medium salinity on the aPDT effects of [Ru(II)] and **26**: relative growth duration (compared to untreated bacteria, in %). The graph from the left to the right report the results obtained for different doses of reagents. Water corresponds to 0X NaCl.

The relationship between salinity and antimicrobial efficacy revealed distinct patterns for [Ru(II)] complex versus aPDT polymers. [Ru(II)] demonstrated concentration-dependent effects, with notable dark toxicity at higher concentrations (50 µg/mL) in water. Its photodynamic activity was most pronounced in water but gradually diminished with increasing salt concentration, suggesting sensitivity to ionic strength.

In contrast, **26** showed a remarkably different profile - exhibiting no dark toxicity even at the highest tested concentration (500 µg/mL) across all salt conditions, while its photodynamic efficacy improved with increasing salinity up to 4X NaCl concentration. This unexpected enhancement of activity in high-salt conditions may result from charge screening effects: as polymer **26** carries negative charges from carboxylic acid groups, it may exhibit lower interaction with the typically negatively charged bacterial cell wall in low ionic strength media through repulsive electrostatic interactions. The addition of salt screens these charges, potentially reducing repulsion and improving polymer-cell interaction, thereby enhancing photosensitizer accessibility and singlet oxygen generation efficiency. The presence of the trithiocarbonate RAFT chain-end in polymer **26** may also contribute to its unique salt-dependent behavior, though the exact mechanism requires further investigation.

To better approximate physiological conditions, studies were extended to artificial sputum medium (ASM), which presents a more complex biological environment containing various biomolecules. In undiluted ASM, photodynamic activity was substantially suppressed for all materials tested. This suppression may result from multiple factors: the medium's complexity potentially interfering with light penetration and singlet oxygen production, the presence of biomolecules that can act as alternative targets for ROS (reducing their availability for bacterial eradication), and the increased viscosity of ASM potentially limiting diffusion of reactive species to bacterial targets. However, when ASM was diluted with water, antimicrobial activity could be partially restored (as detailed in Figure 53) demonstrating the significant impact of environmental factors on aPDT efficacy.

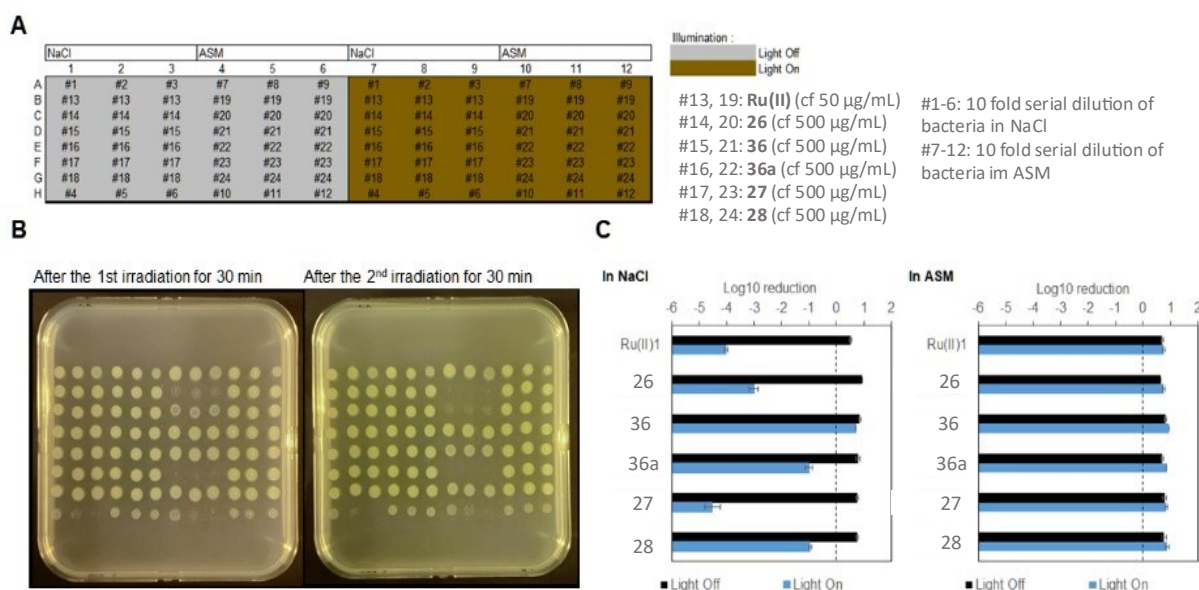


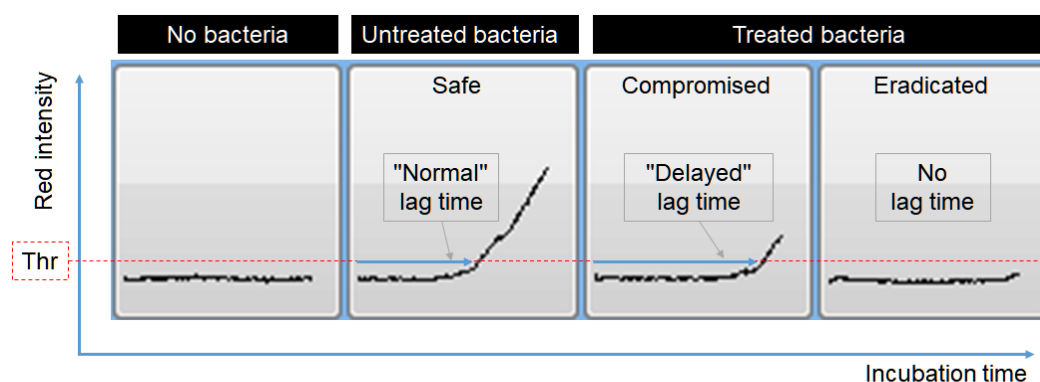
Figure 53: Antibacterial assay in 1X NaCl or an artificial sputum medium (ASM) using planktonic cells of a *P. aeruginosa* strain (at exponential growth phase). Exposure to light was done two times for 30 min each (prolonged irradiation treatment). Layout of the experiment (A), growth of replicates done on LB agar plates after the first and second exposure to light (B) and calculation of Log₁₀ CFU reduction after the latter (C).

These findings demonstrate that polymer charge characteristics critically influence aPDT efficacy under physiologically relevant conditions: while positively charged polymers (**36**, **36a**, **37**) show salt-sensitive activity, negatively charged polymers (**27**, **33**) maintain effectiveness in high-salt environments, positioning them as lead candidates for applications in complex biological settings such as respiratory infections.

3.4.4 Assays using Bacteria in Biofilms

The efficacy of [Ru(II)] and aPDT polymers against bacterial biofilms was evaluated through metabolic activity assays using *P. aeruginosa* strains (CH2678 and PA19). This assessment was particularly crucial as biofilms represent a significant therapeutic challenge, displaying up to 1,000-fold higher resistance to antibiotics compared to their planktonic counterparts.[145] This dramatically enhanced resistance stems from multiple factors: the protective barrier of the extracellular matrix that impedes drug penetration, the presence of metabolically dormant cells within the biofilm structure, and altered bacterial metabolic states.[146] These protective mechanisms typically necessitate substantially higher antibiotic concentrations for effective treatment, often exceeding clinically achievable levels.[27,28,129]

The metabolic activity of treated biofilms was evaluated using triphenyl tetrazolium chloride (TTC) as a metabolic indicator. When metabolically active biofilm cells reduce TTC, it forms 1,3,5-triphenylformazan, which appears red in color.[147] The intensity and timing of this color change provides information about biofilm viability: untreated biofilms show a 'normal' lag time before color development, while compromised biofilms show delayed color development, and eradicated biofilms show no color change (Figure 54).



Thr = threshold, i.e., the intensity above which the red coloration is considered to be significantly above the background, denoting metabolic activity.

Figure 54: Schematic representation of TTC-based metabolic activity assay. The graphs show red intensity over incubation time for different biofilm conditions: no bacteria (negative control), untreated bacteria showing normal lag time, treated bacteria showing compromised biofilms with delayed lag time, and eradicated biofilms showing no metabolic activity.

In this assay, a longer time to detect red coloration indicates more effective suppression of biofilm metabolic activity. Under dark conditions (grey), none of the aPDT materials showed significant impact on biofilm metabolism, consistent with their

photodynamic mechanism of action. However, upon light exposure (light blue), marked differences in antibiofilm efficacy emerged among the different aPDT compounds, details are shown in Figure 55.

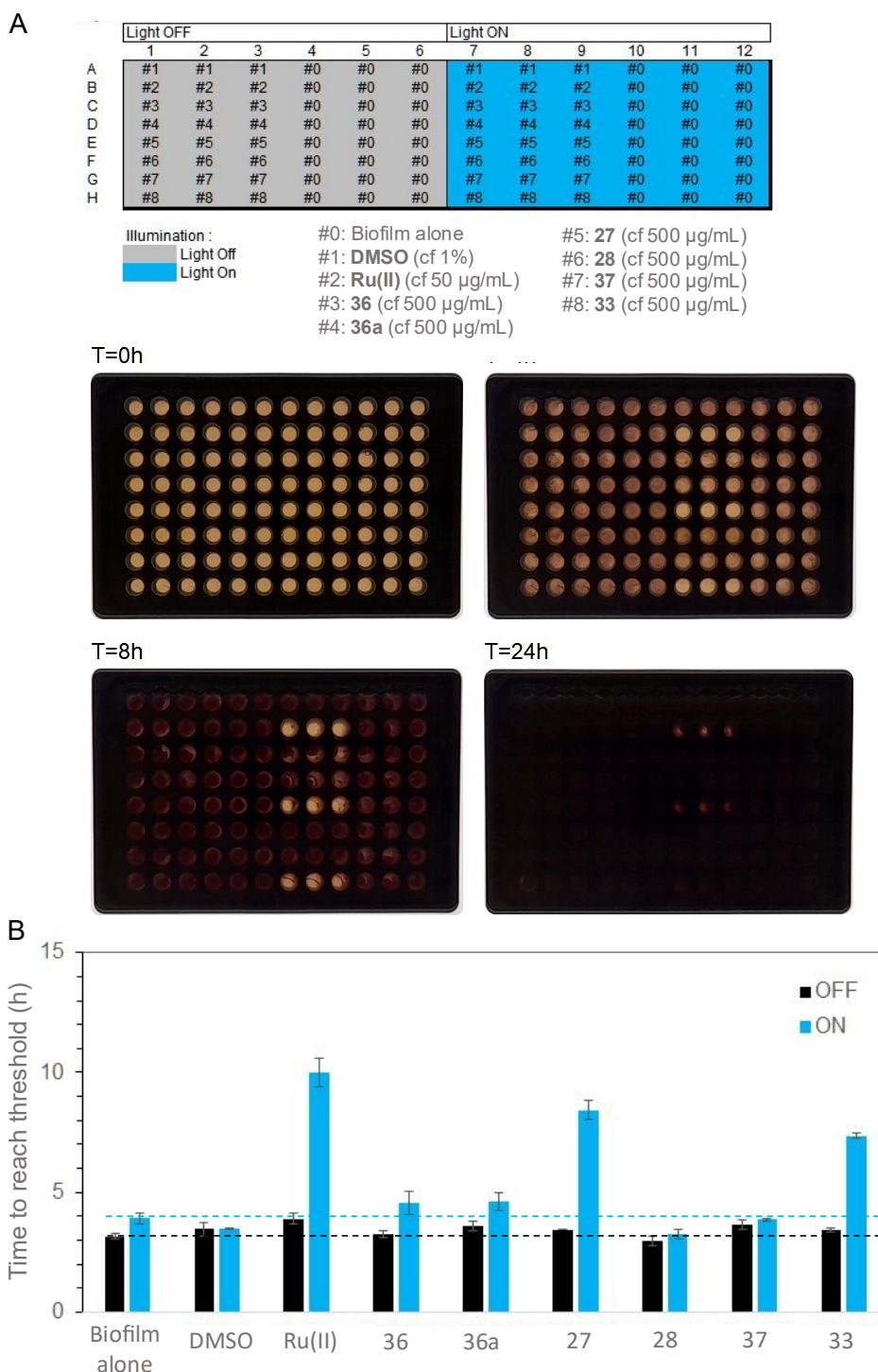


Figure 55: Metabolic activity of CH2678 biofilms treated with Ru(II) or aPDT polymers and kept in the dark (grey) or exposed to light (light blue): pictures of the biofilms at different incubation times after treatment (A) and time needed for detecting metabolic activity (red shifting). (B) [Ru(II)] and aPDT polymers were tested at 50 and 500 µg/mL, respectively. The dashed lines indicate the level determined for biofilm alone (not exposed to any compound). The data correspond to mean $\pm$ SD with $n=3$.

Notably, **27** and **33** demonstrated superior antibiofilm activity compared to other aPDT polymers, requiring substantially longer times (approximately 8-10 hours) for metabolic activity detection. This enhanced efficacy was particularly interesting given their previously observed slower kinetics of singlet oxygen production, suggesting that sustained photodynamic action might be advantageous for biofilm eradication. In contrast, **28** showed no significant antibiofilm activity under any tested condition, maintaining activity detection times similar to untreated biofilms.

The impact of environmental conditions on antibiofilm activity was further investigated by comparing treatments in water versus saline (0.9% NaCl) across a range of concentrations, details are shown in Figure 56. The response patterns revealed concentration-dependent effects that varied among the different materials. In water, both **27** and **33** maintained their superior efficacy across a broad concentration range. Interestingly, in saline conditions, these polymers showed even more pronounced antibiofilm effects, with lag times extending beyond 15 hours at higher concentrations. This enhanced activity in physiological salt conditions is particularly relevant for potential therapeutic applications.

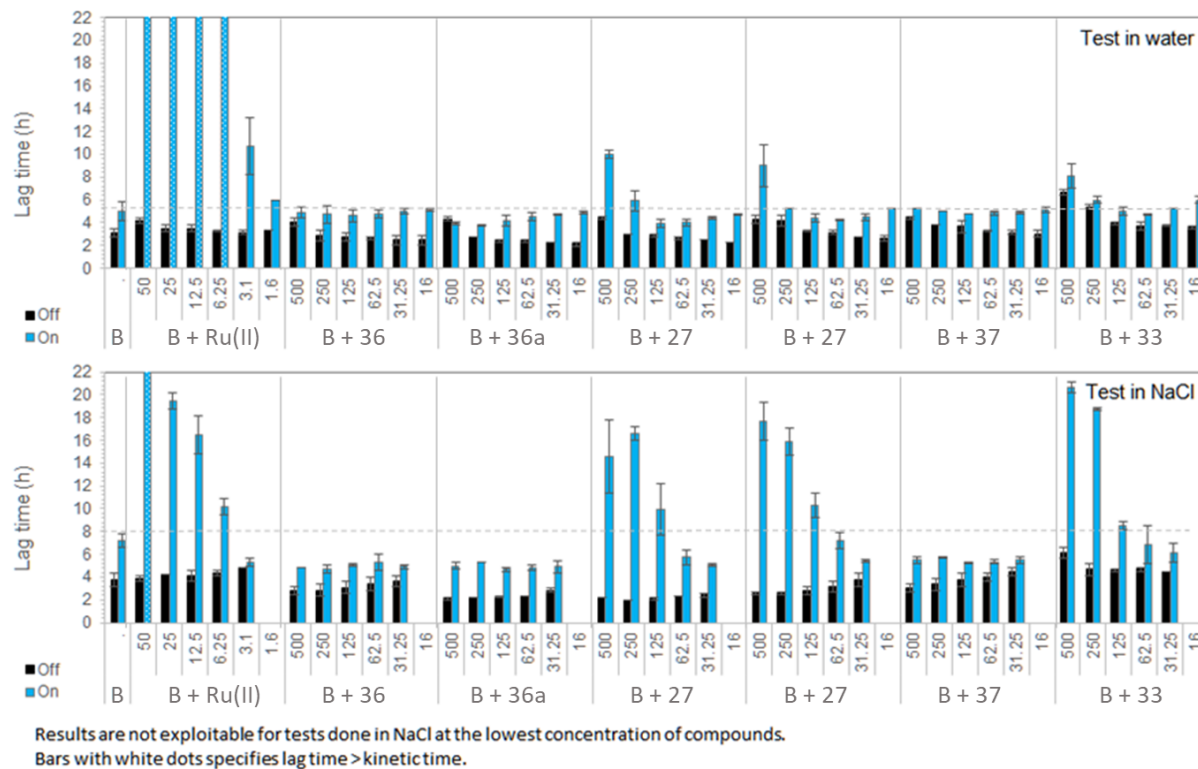


Figure 56: Test of PA19660 biofilm (2 days old): lag time during which no red coloration was detected after biofilms were treated with a compound and exposed (“on”) or not (“off”) to light. The dashed line indicates the level determined for biofilm alone (not exposed to any compound). On the x axis, the letter

'B' stands for biofilm and the values refer to the concentrations of a compound (in $\mu\text{g/mL}$), at various concentrations obtained by dilutions done in water or in 0.9% NaCl. Each bar corresponds to the mean of at least 2 biofilms = 2 technical replicates ($n \geq 2$ +/- SD).

The differential effectiveness of aPDT polymers against biofilms compared to planktonic cells, particularly for **27** and **33**, suggests distinct mechanisms of action that may be influenced by the biofilm environment. Their maintained or enhanced activity in saline conditions further highlights their potential utility in physiologically relevant settings.

Alternative polymers without PS to disrupt biofilms

A parallel investigation examined an alternative strategy utilizing polymers incorporating established biofilm-disrupting agents (farnesol, agmatine, L-arginine, and thymol) at 5 mol% loading. The structures of the polymers are shown in Figure 57. These HEAAm-based polymers, containing no photosensitizer, were synthesized by the same strategies used to make the aPDT polymers discussed above (FRP and PPM). These experiments serve as reference polymers to understand the baseline activity of biofilm-disrupting motifs alone, as these agents are intended to augment the efficiency of aPDT polymers in future combined formulations.

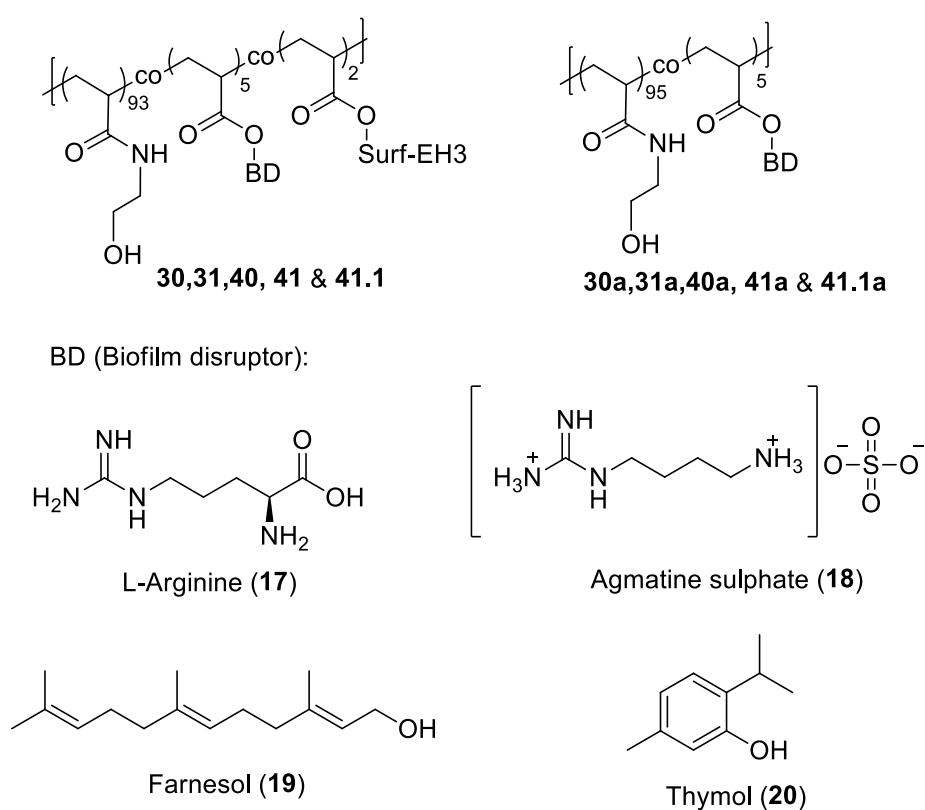


Figure 57: General structure of polymers containing biofilm-disrupting agents (BD) without photosensitizers. Polymers **30/30a** contain agmatine; **31/31a** contain L-arginine; **40/40a** contain farnesol; **41/41a/41.1/41.1a** contain thymol. Variants differ in surfactant content (designated 'a') and BD loading (1 or 5 mol%, indicated by *).

The materials were tested against *P. aeruginosa* biofilms to evaluate both prevention of biofilm formation and disruption of established biofilms. Biomass was quantified using crystal violet (CV) staining, which measures total biofilm including extracellular matrix and cells, while metabolic activity was assessed using triphenyl tetrazolium chloride (TTC) reduction, which indicates viable bacterial cells.

Figure 58 shows two experimental conditions: the top panels present tests on preformed biofilms (biofilms were established for 2 days, then treated with compounds), while the bottom panels show tests during biofilm formation (compounds were present from the start of bacterial culture).

Analysis of individual compounds revealed:

- Free biofilm disruptors (agmatine #1, farnesol #4, L-arginine #7, thymol #10) showed minimal to no effect on either pre-formed biofilms or biofilm formation at the tested concentrations, with both CV and TTC absorbance remaining near untreated control levels (dashed line at 1.0).
- Biofilm disruptor-containing polymers (#2, #3, #5, #6, #8, #9, #11-#14) demonstrated concentration-dependent effects. Thymol-containing polymers (#11-#14) showed the most notable reduction, particularly in preventing biofilm formation at higher concentrations (≥ 100 $\mu\text{g/mL}$). However, even these polymers achieved only modest reductions (10-30% decrease) compared to controls.
- Pre-formed biofilms (top panels) were generally more resistant to treatment than biofilms forming in the presence of disruptors (bottom panels), consistent with the protective nature of established biofilm matrices.

The yellow shaded region in Figure 58 represents the background range of bacterial growth and metabolic activity in untreated controls. Values within this range indicate that compounds have minimal effect on bacterial growth or biofilm formation. Antibiotic controls (Gentamycin G, Tobramycin T) showed strong activity with values well below the background range, demonstrating effective biofilm control. In contrast, most biofilm disruptor samples (#1-14) remained within or near the yellow zone, confirming their expected limited antimicrobial activity in the absence of photodynamic activation.

Overall, these materials without antimicrobial agents demonstrated limited efficacy in both preventing biofilm formation and disrupting established biofilms when tested without photodynamic activation. These results highlight the comparative advantage of our photodynamic approach, which achieves more substantial biofilm control through light-activated mechanisms, yet in future experiments the combined activity of biofilm-disruptor polymers with aPDT polymers needs to be evaluated to assess any synergetic advantage.

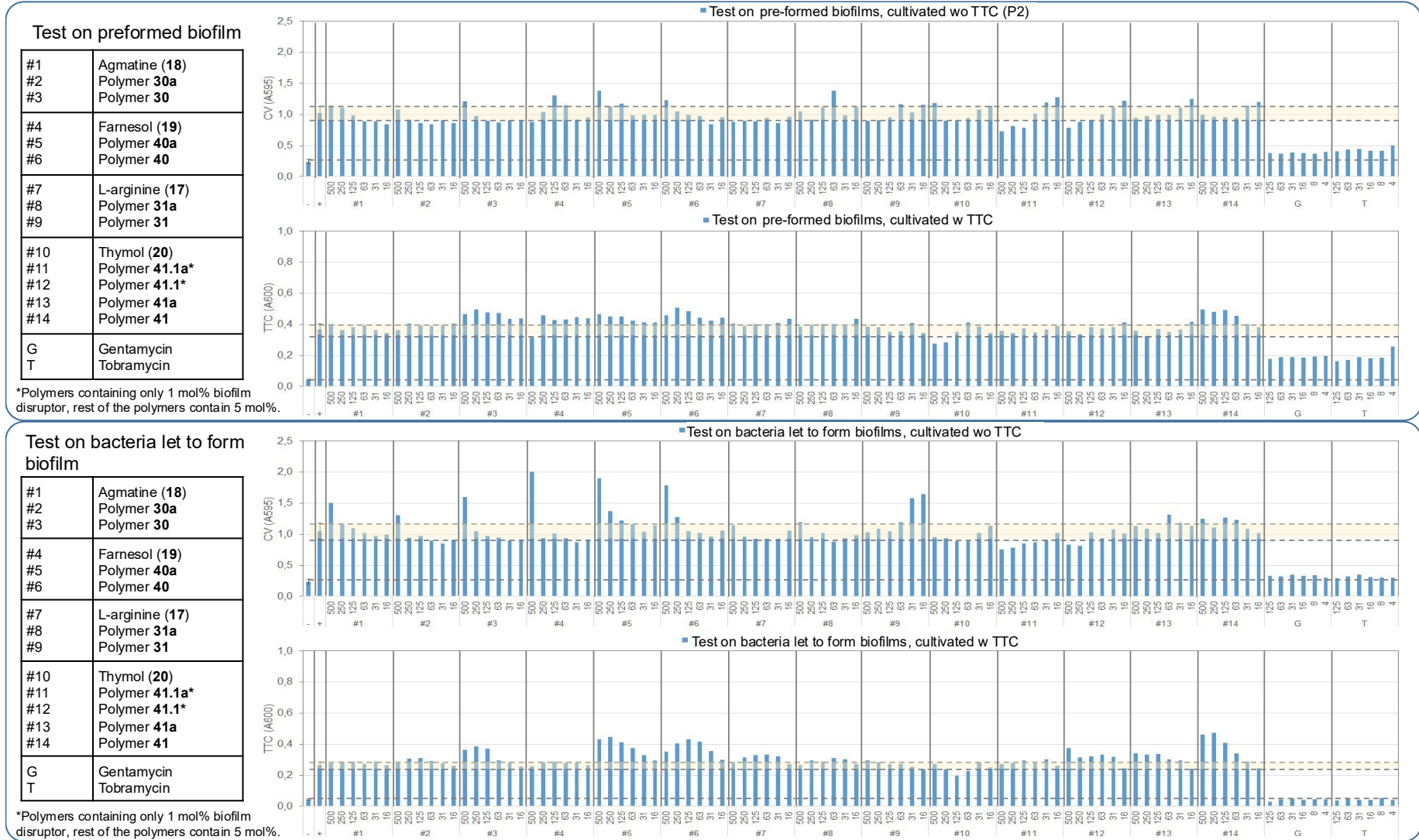
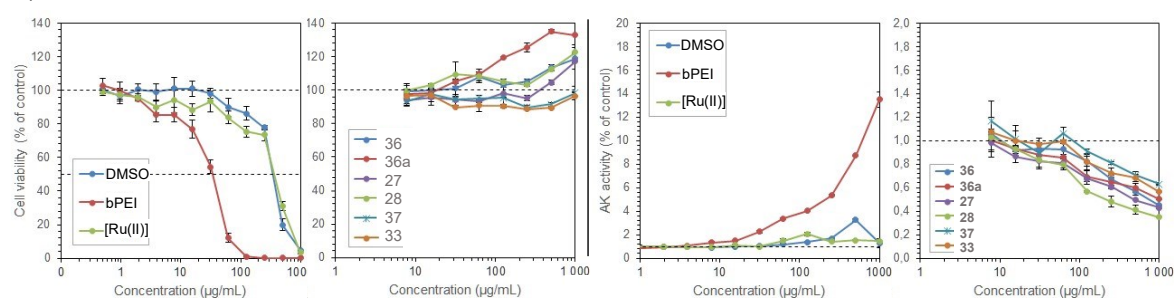


Figure 58: Evaluation of biofilm-disrupting polymers against *P. aeruginosa*. Sample identities (left). Crystal violet (CV) and triphenyl tetrazolium chloride (TTC) absorbance for preformed biofilms (top) and during biofilm formation (bottom). Yellow region indicates untreated control range.

3.4.5 Assays using Eukaryotic Cells

The biocompatibility assessment of aPDT polymers employed two human bronchial epithelial cell lines, 16HBE and A549, to evaluate potential effects on human tissues. The analysis incorporated complementary methodologies, measurement of cellular ATP content to assess metabolic activity, and monitoring of adenylate kinase (AK) release to detect membrane damage, details are shown in Figure 59. This dual approach provided comprehensive insights into both acute cytotoxicity and subtle metabolic effects.

A) Test of 16HBE



B) Test of A549

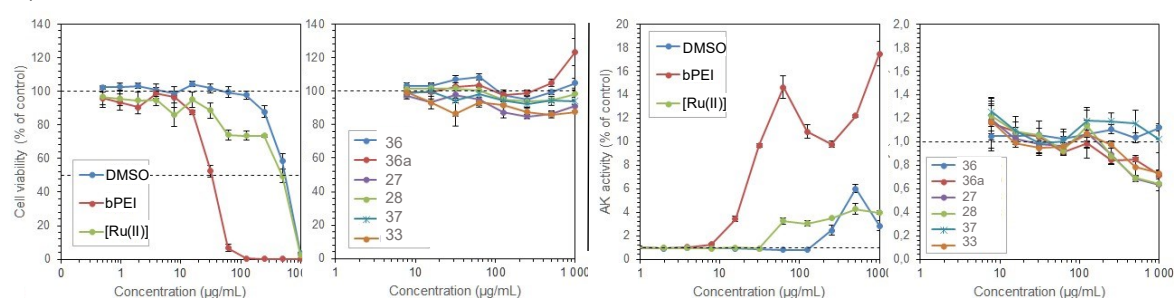


Figure 59: Side-effects towards eukaryotic cells after exposure for 24h to Ru(II) or aPDT polymers: evaluation using 16HBE (A) and A549 (B) epithelial cells. Viability was determined considering the ATP content of cells and cytotoxicity was determined by measuring the leakage from cells of adenylate kinase (AK). As controls, DMSO (vehicle used with Ru(II)) and branched PEI (bPEI) were tested in parallel. The dashed lines refer to 100% and 50% cell viability or to 1 AK activity. The data correspond to mean $\pm$ SD with $n=3$

The toxicity screening included several reference materials to establish comparative baselines. Branched polyethylenimine (bPEI, 25 kDa) was included as a known cytotoxic polymer, while DMSO served as the vehicle control for [Ru(II)] complex evaluations. The free [Ru(II)] complex itself was also tested to understand the impact of polymer conjugation on cytotoxicity.

Among the tested materials, bPEI demonstrated the most severe cytotoxic effects on both cell lines. Under the experimental conditions, bPEI exhibited an LD50 (lethal dose causing 50% cell death) between 30-60 $\mu\text{g/mL}$, with significant cell membrane

damage evidenced by elevated AK levels at concentrations as low as 16-30 µg/mL. The [Ru(II)] complex showed intermediate toxicity, with an LD50 ranging from 250-500 µg/mL, though this effect was partially attributable to its DMSO vehicle, which displayed dose-dependent cytotoxicity in control experiments.

In marked contrast to these reference materials, all aPDT polymers exhibited remarkable biocompatibility across both cell lines. The polymers maintained cell viability and showed no significant AK release even at concentrations exceeding 1,000 µg/mL - the highest concentration tested in the study. This exceptional tolerance threshold is particularly noteworthy as it substantially exceeds the concentrations required for effective antimicrobial activity, establishing a wide therapeutic window. The robustness of these findings was further validated through additional testing with CFBE cells, which yielded consistent results.

This clear separation between antimicrobial efficacy and eukaryotic cell toxicity has significant implications for therapeutic applications, particularly in respiratory contexts where selective antimicrobial activity must be achieved while preserving bronchial epithelial cell integrity. The demonstrated safety profile of these aPDT polymers, combined with their effective antimicrobial properties, positions them as promising candidates for further development.

3.4.6 Nebulization Studies

The potential application of these materials for treating respiratory infections necessitated evaluation of their compatibility with nebulization-based delivery systems. A systematic assessment of structural integrity and functional properties before and after nebulization was performed using an eFlow mesh nebulizer (Pari, Germany). Polymer solutions were nebulized and samples collected before and after the process for comparative analysis of UV-visible absorption, phosphorescence emission, electrophoretic mobility, and antimicrobial activity against *P. aeruginosa*. This evaluation provided critical insights into their stability during aerosolization.

Spectroscopic analysis demonstrated that all aPDT polymers maintained their structural integrity through the nebulization process, as evidenced by consistent UV-visible absorption profiles before and after nebulization, as shown in Figure 60. Emission measurements showed minimal changes in emission intensity, indicating preservation of the ruthenium complex's photophysical properties. The maintenance of electrophoretic mobility patterns after nebulization further confirmed the structural stability of these materials during the aerosolization process.

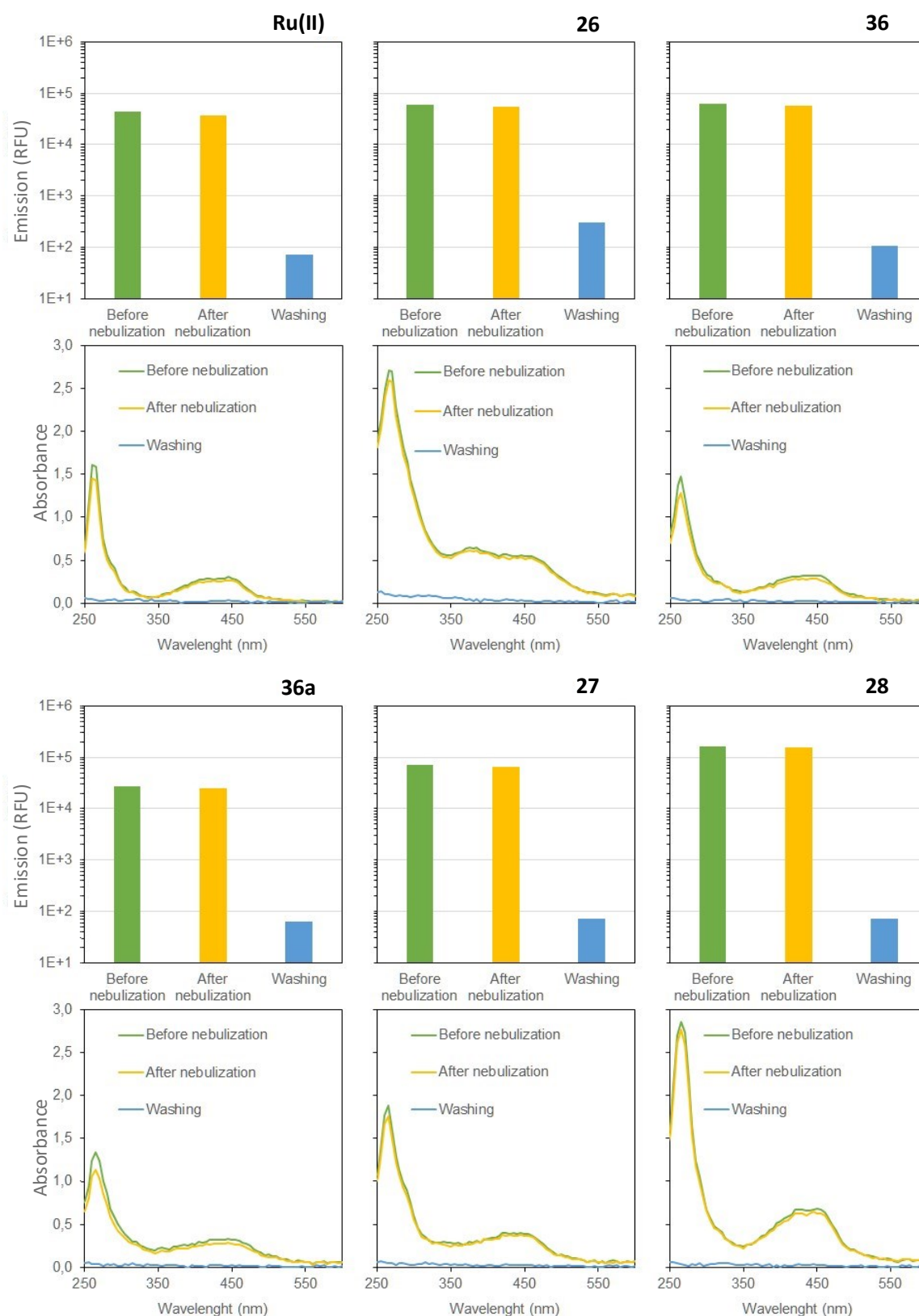


Figure 60: Emission intensity (top panels) and UV-visible absorption spectrum (bottom panels) of Ru(II) complex and aPDT polymers before or after nebulization. “Washing” corresponds to a control performed with water to check the washing efficiency of the nebulizer device that was done between each compound nebulized.

The spectroscopic analysis revealed consistent profiles before and after nebulization, confirming retention of the photophysical properties of the materials. The retention of structural integrity was corroborated by electrophoretic profiling, with details shown in Figure 61 and Figure 62.

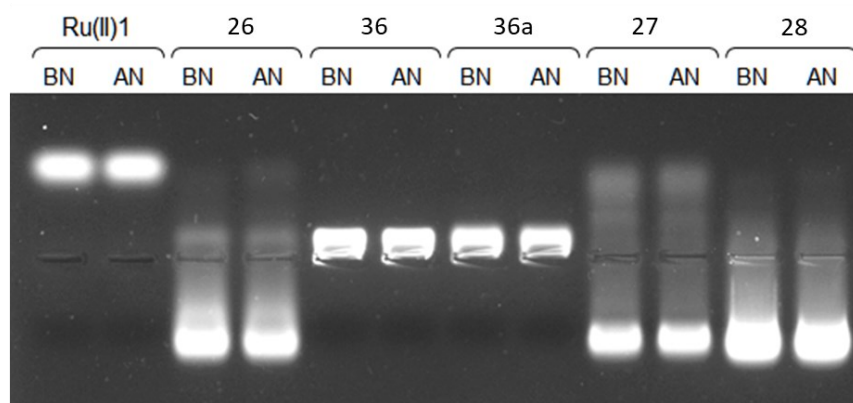


Figure 61: Electrophoretic profiles of [Ru(II)] complex and aPDT polymers before (BN) and after (AN) nebulization using an eFlow mesh nebulizer. Consistent band positions and intensities indicate preservation of polymer structural integrity during the aerosolization process.

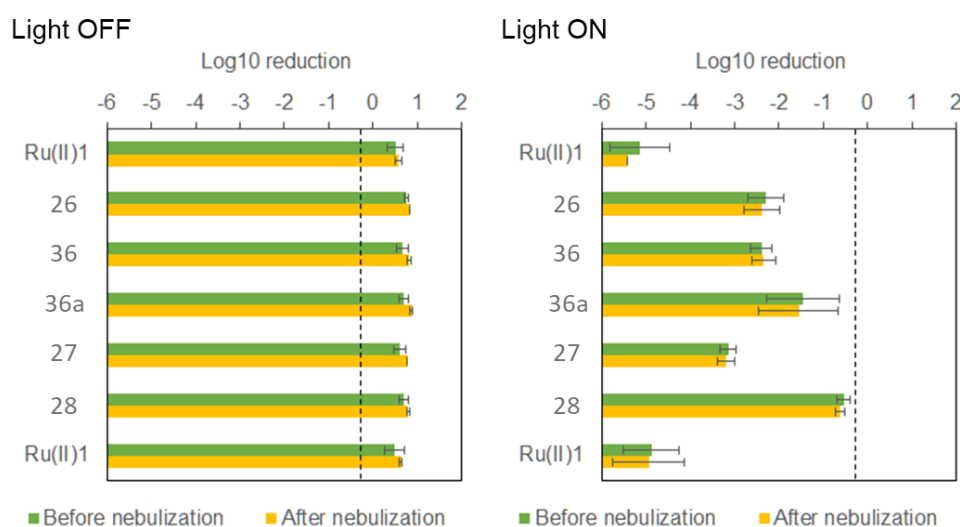


Figure 62: Antimicrobial activity of [Ru(II)] complex and aPDT polymers before and after nebulization. *P. aeruginosa* CFU $\log_{10}$ reduction upon light irradiation (450 nm, 30 min) shows preserved photodynamic efficacy post-nebulization. Light OFF: no irradiation (dark control); Light ON: after light exposure. Mean $\pm$ SD with $n=3$.

Most importantly, bacterial assays using the nebulized materials demonstrated preservation of their photodynamic antimicrobial activity, with post-nebulization samples achieving similar levels of bacterial reduction as pre-nebulization materials. This retention of biological activity through the nebulization process is particularly significant for potential therapeutic applications in treating respiratory infections, where aerosol delivery would be the preferred administration route.

Summary of the biological results

The comprehensive biological evaluation of the synthesized antimicrobial photodynamic polymers revealed several promising candidates with potent light-activated antimicrobial activity independent of the antibiotic resistance profile of the strains, along with favorable biocompatibility profiles. The systematic testing approach provided valuable insights into the structure-activity relationships and the influence of ionic strength (NaCl concentration), medium complexity (artificial sputum medium), and bacterial growth state (planktonic vs. biofilm) on the materials' performance. Initial characterization of the polymers' charge properties through electrophoresis demonstrated the impact of synthesis method and composition on their interactions with bacterial cells and biomolecules like DNA. Quantification of singlet oxygen production, a key mediator of photodynamic activity, showed material-dependent and medium-dependent effects, with some polymers exhibiting enhanced activity under physiological salt conditions.

Antimicrobial efficacy studies against planktonic bacteria revealed the light-dependent nature of the polymers' activity, with clear differences observed between Gram-negative and Gram-positive species. Notably, certain polymers (**27** and **33**) maintained their efficacy even in complex media and high salinity, suggesting potential for application in physiologically relevant settings. The ability to achieve spatial control over bacterial inactivation through targeted light delivery was also demonstrated, highlighting the precision and versatility of the photodynamic approach.

Evaluation of the polymers' antibiofilm activity uncovered their potential for tackling this challenging form of bacterial growth. The efficacy of **27** and **33** against biofilms, particularly in saline conditions, is in agreement with their performance against bacteria in the planktonic state and distinguished them as promising candidates for further development. Comparison with alternative non-photodynamic strategies using biofilm-disrupting agents confirmed the advantages of the light-activated approach. While the polymeric biofilm disruptors showed limited activity in the absence of antimicrobial agents, their intended role is to augment aPDT efficacy when combined with photosensitizers in dual-functional systems. This combinatorial strategy, currently under investigation in our group, (PhD work of Jannik Frynn) aims to leverage

synergistic mechanisms: biofilm disruption to enhance photosensitizer penetration and light delivery, coupled with photodynamic bacterial elimination.

Biocompatibility studies with human bronchial epithelial cells demonstrated the polymers' excellent safety profile, with minimal toxicity observed even at concentrations far exceeding those required for antimicrobial efficacy. This wide therapeutic window is a key advantage for potential clinical applications, especially for managing recalcitrant pulmonary infections in patients with medical conditions such as cystic fibrosis.

Finally, the successful nebulization of the polymers without compromising their structural integrity or antimicrobial activity confirmed their suitability for potential pulmonary delivery, a major step towards developing treatments for respiratory infections.

In conclusion, the biological evaluation identified lead polymers with potent, spatially controlled antimicrobial activity against both planktonic and biofilm forms of bacteria, while maintaining excellent biocompatibility and nebulization stability. These findings underscore the potential of antimicrobial photodynamic polymers as a promising therapeutic platform to tackle the spread of antibiotic-resistant microorganisms and lay the foundation for future translational development.

4 Conclusion and Outlook

Conclusion

The primary objective of this thesis was to develop innovative antimicrobial photodynamic polymers for potential treatment of bacterial infections, with a particular focus on respiratory diseases. Two complementary synthetic strategies were successfully established: post-polymerization modification (PPM) via active ester chemistry and direct free radical polymerization (FRP).

These approaches enabled controlled incorporation of photosensitizers (ruthenium complexes and phenalenone) and biofilm-disrupting agents into biocompatible polymer systems suitable for respiratory delivery.

Comprehensive biological evaluation identified polymers **27** and **33** as lead candidates. These materials demonstrated potent light-activated antimicrobial activity against both planktonic *P. aeruginosa* and biofilms, with efficacy maintained or enhanced under physiologically relevant conditions including high salinity and artificial sputum medium. Importantly, both polymers exhibited excellent biocompatibility with human bronchial epithelial cells (no toxicity up to >1000 µg/mL) while maintaining antimicrobial activity at 10-100-fold lower concentrations, establishing a favorable therapeutic window.

Structural integrity and photodynamic function were preserved following nebulization, confirming compatibility with aerosol delivery systems.

This work establishes a foundation for polymer-based antimicrobial photodynamic therapy targeting respiratory infections. The combination of effective bacterial elimination, physiological robustness, and delivery compatibility demonstrates the feasibility of this approach for treating antibiotic-resistant pulmonary pathogens.

Outlook

To further advance the development of the lead polymers identified in this work, several key steps are envisioned. Additional optimization of polymer composition and photosensitizer loading could enhance their antimicrobial efficacy, while more detailed mechanistic studies would elucidate the mode of action and structure-activity relationships governing their performance. Expanding the testing to a broader panel of clinically relevant bacterial strains, including multi-drug-resistant isolates, will provide a more comprehensive assessment of their therapeutic potential.

A particularly promising direction is the development of dual-functional polymers combining both photosensitizers and biofilm-disrupting agents within a single polymer system. While this thesis demonstrated the independent activities of aPDT polymers and biofilm-disrupting polymers, their synergistic combination could significantly enhance antimicrobial efficacy, particularly against biofilms. This strategy aims to leverage biofilm disruption to improve photosensitizer penetration and light delivery into the biofilm matrix, while simultaneously eliminating bacteria through photodynamic action. This combinatorial approach is currently being investigated in the ongoing PhD work of Jannik Fryn, which focuses on optimizing the ratios and incorporation methods of both functional components to maximize antibiofilm activity.

Future studies should focus on evaluating the polymers' performance in more complex biological models, such as ex vivo lung tissue and in vivo animal models of respiratory infection. These investigations will validate their efficacy and safety in a more representative environment and pave the way for clinical translation. Addressing potential challenges, such as formulation development for optimal pulmonary delivery and designing appropriate light delivery systems for in situ irradiation of the lung, will be critical for successful implementation.

In parallel, conducting thorough toxicology and pharmacokinetic studies will establish the safety profile of these polymers and guide their clinical development. Exploring broader applications beyond respiratory infections, such as in wound healing or medical device coatings, could expand the impact of this work and address other areas where antimicrobial and biofilm-disrupting properties are valuable.

Finally, investigating the potential for combining antimicrobial photodynamic therapy with other therapeutic approaches, such as antibiotics or immunomodulators, may

lead to enhanced efficacy and prevention of resistance development. By harnessing the synergistic effects of multiple treatment modalities, more effective and sustainable solutions to the global challenge of antimicrobial resistance could be achieved.

In conclusion, the antimicrobial photodynamic polymers developed in this thesis hold great promise as a novel therapeutic platform. With further development and translation, this work has the potential to significantly improve patient outcomes and contribute to the fight against the growing threat of antimicrobial resistance. The stage is set for an exciting future in this field, where innovative materials and strategies will play a crucial role in safeguarding public health.

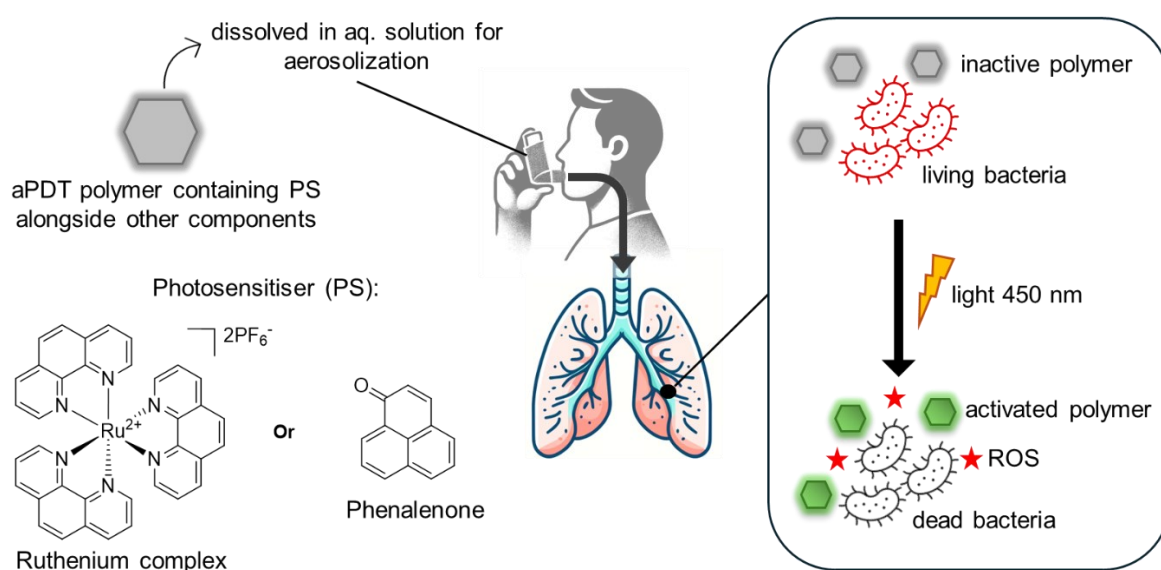


Figure 63: Proposed therapeutic strategy for polymer-based antimicrobial photodynamic therapy in respiratory infections. aPDT polymers containing photosensitizers are delivered via nebulization, followed by targeted light activation to generate reactive oxygen species for bacterial elimination.

5 Experimental Section

5.1 Chemicals

Acryloyl chloride (96%, stabilized with 400 ppm phenothiazine, Alfa Aesar), Aliquat 336, 2-aminoethanethiol hydrochloride (> 98%, Alfa Aesar), 2-aminoethanol (> 98%, Alfa Aesar), 3-phenyl-2-propenoyl chloride (Cinnamoyl chloride, 97%, BLDpharm), *N,N'*-dicyclo-hexylcarbodiimide (DCC, Fluka), 4-dimethylaminopyridine (DMAP, recrystallized from EtOH), 5,6-epoxy-5,6-dihydro-[1,10]phenanthroline (95%, BLDpharm), 1,10-phenanthroline (pro analysi, Merck), 1,10-Phenanthroline-5-amine (98%, Aldrich), pentafluorophenol (abcr GmbH, 99%), farnesol (95%, Merck), formalin (37% aqueous solution), glycerine, hydroquinone (for synthesis, Merck-Schuchardt), naphthalene (99%, Acros Organics), 2-naphthol, paraformaldehyde, sodium acrylate (97%, Aldrich), thymol ($\geq$ 98%, Alfa Aesar), trifluoroacetic acid (TFA, for synthesis, Sigma-Aldrich) and triethylamine (Et₃N, puriss., $\geq$ 99.0%, distilled and stored under argon, ChemSolute) were used.

The solvents acetone (100%, VWR), chloroform ($\geq$ 99.8%, Fisher Chemical), dichloromethane (technical, $\geq$ 98%, stabilized with 0.2% ethanol, VWR), diethyl ether (Et₂O, pro analysi, 99.5%, Thermo Scientific), dioxane, dimethylformamide (DMF, 100.00%, VWR), dimethyl sulfoxide (DMSO, 99.98%, Fisher Chemical), ethanol (absolute, VWR), ethanol (EtOH, 99%, denatured, bioethanol, ChemSolute), ethyl acetate (analytical grade, $\geq$ 99.8%, Fisher Chemical), hexane (mixture of isomers, VWR), methanol (MeOH, technical, $\geq$ 98.5%, VWR), and toluene (analytical reagent grade, $\geq$ 99.8%, Fisher Chemical) were used as they were received from the manufacturer. Tetrahydrofuran (THF) was dried in a circulation apparatus with potassium under argon atmosphere.

Glacial acetic acid (pro analysi, min. 99.5%, ChemSolute), hydrochloric acid (HCl, 37%, Fisher Chemical), ortho-phosphoric acid (puriss., 85%, Riedel-de Haën), and sulfuric acid (analytical reagent grade, $\geq$ 95%, Fisher Chemical) were used as they were received from the manufacturer.

Ammonium hexafluorophosphate, aluminium chloride (anhydrous, BASF), lithium chloride (99%, anhydrous, Acros Organics), magnesium sulphate (puriss., $\geq$ 99%, Carl Roth), potassium carbonate (puriss., Bernd Kraft), ruthenium (III) chloride (RuCl₃ × 3H₂O, Merck), sodium hydrogen carbonate (sodium bicarbonate, $\geq$ 99.7%,

ChemSolute), sodium hydroxide (99.20%, VWR), sodium-3-nitrobenzolsulfonate (Merck), and sodium sulphate (anhydrous, VWR) were used as they were received from the manufacturer.

The main monomer *N*-hydroxyethyl acrylamide (HEAAm, 97%, stabilized with 1000 ppm hydroquinone monomethyl ether, Sigma-Aldrich) was stored at 5 °C. The acrylic acid (for synthesis, stabilized with hydroquinone monomethyl ether, Merck) and the [2-(Acryloyloxy)ethyl]trimethylammonium chloride solution (80 wt% in H₂O, Aldrich) were also used as they were received. The surfactant monomer EH3A (FRJ045, 90%) was synthesized in an earlier work by Jannik Fryn and was stored in the freezer. 2-(ethylthiocarbonothioylthio)-2-methyl propanoic acid (EMP) and 2-[(Dodecylthio)carbonothioyl]thio-2-methylpropanoic acid (DMP) were synthesized previously in the Macromolecular working group. (1-Oxo-1H-phenalen-2-yl) methanaminium chloride (PN-NH₃Cl) was provided by Dr. Jérémy Godard Université Paris Cité (UPC). The initiator 4,4'-azobisisobutyronitrile (AIBN, 99%, Acros Organics) was recrystallized from methanol and acetone and stored at 5 °C in the absence of light.

The solvents used for the ¹H NMR spectra were deuterated acetone ((CD₃)₂CO, 99.8%, Deutero) and deuterated chloroform (CDCl₃, 99.8%, Deutero), both stored at 5 °C. Deuterated dimethyl sulfoxide ((CD₃)₂SO, 99.8%, Deutero) was stored under argon at room temperature with a 4 Å molecular sieve.

Deionized water was obtained using a Milli-Q Direct-Q 3UV water purification system (conductivity σ = 18.2 MΩ cm).

For the dialysis of the polymers dialysis tubes (Spectra/Por 6 Dialysis Membrane, pre-wetted RC tubing, MWCO: 1 kDa / 3.5 kDa, Spectrum) were used.

The syringe filters used (0.22 μm / 0.45 μm, PTFE) were manufactured by ChromPure.

5.2 Instruments

Gel-Permeation-Chromatography (GPC): was used to measure the molar mass distributions and dispersities of the polymers. For DMAc-soluble polymers, a PSS-SECcurity system Agilent Technologies 1260 infinity was employed, equipped with a PSS GRAM 100Å column and a 10 µm particle size pre-column. The eluent was a solution of DMAc with 0.1% LiBr. A sample volume of 20 µL was injected at a temperature of 60 °C, with a flow rate of 1 mL/min. The calibration curve was generated using PMMA standards from Polymer Standard Service, Mainz, and the molar mass of the polymers was calculated using the Mark-Houwink-Constants.

For water-soluble polymers, a PSS-SECcurity system Agilent Technologies 1200 Series was used, equipped with a PSS Suprema 3000 Å column and a 10 µm particle size pre-column. The eluent in this case was a mixture of water/MeCN (85:15 vol%) with NaNO₃ (0.1 mol·L⁻¹) and NaN₃ (0.011 mol·L⁻¹). The sample volume and flow rate were the same as for the DMAc-soluble polymers, but the temperature was set at 30 °C. Pullulan standards from Polymer Standard Service, Mainz were used for calibration, and the molar mass was again calculated using the Mark-Houwink-Constants.

NMR measurements were conducted using a Bruker Avance 400 and/or Jeol ECZ 500 spectrometer. ¹H NMR spectra were recorded at frequencies of 300 MHz, 400 MHz, and 500 MHz, while ¹³C NMR measurements were performed at 126 MHz. All spectra were acquired at room temperature (20 °C).

The chemical shifts were reported in parts per million (ppm), using the signals of non-deuterated solvents as internal standards. The following internal standards were used for different solvents:

CDCl₃: ¹H at 7.26 ppm, ¹³C at 77.2 ppm.

Aceton-*d*₆: ¹H at 2.75 ppm, 2.92 ppm, and 8.03 ppm.

D₂O: ¹H at 4.75 ppm.

DMSO-*d*₆: ¹H at 2.50 ppm, ¹³C at 39.52 ppm.

MeOD-*d*₄: ¹H at 3.31 ppm, ¹³C at 49.0 ppm.

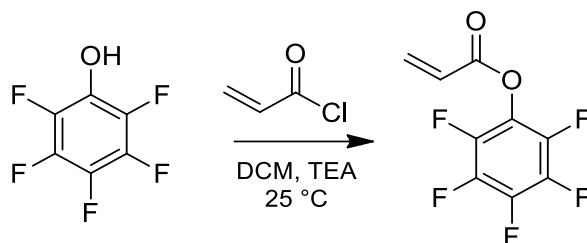
Signal assignment was performed using MestReNova.

Biochemical Part: The descriptions, instrumentation, materials and procedures of the biochemical experiments were performed in the laboratories of Prof. Dr. Tristan Montier/Dr Tony Le Gall from the university of Brest (F).

5.3 Synthesis of Active Ester Monomers

Pentafluorophenolacrylate (PFPA) (**21**)

The reaction was carried out according to a modified procedure from literature.[117,148]



Pentafluorophenol (2 g, 10.9 mmol) was dissolved in 30 ml of dichloromethane (DCM) in an ice bath. Triethylamine (TEA) (1.5 ml, 11 mmol) was then added. Acryloyl chloride (0.9 ml, 11 mmol) was diluted in 20 ml of DCM and added dropwise to the mixture. The reaction was stirred and allowed to warm to room temperature overnight. Thin-layer chromatography (TLC) developed in DCM indicated the presence of the product in the clear solution. The mixture was washed three times with water and twice with brine, then dried over anhydrous sodium sulfate (Na₂SO₄). Evaporation of the solvent yielded a colorless liquid with a very strong odor.

Yield: 64%, 1.76 g, 7.4 mmol.

Spectral data:

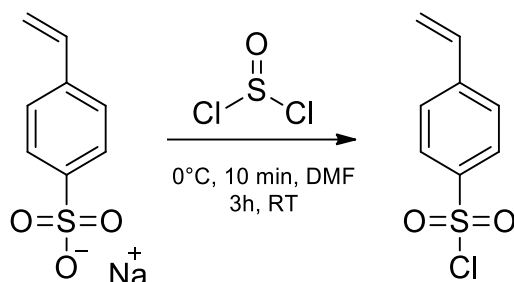
¹H NMR (500 MHz, CDCl₃), δ ppm: 6.76 – 6.64 (m, 1H, -CH=), 6.44 – 6.29 (m, 1H, =CH₂), 6.23 – 6.07 (m, 1H, =CH₂).

NMR spectra is found in Fig. SI 1 in appendix.

Synthesis of pentafluorophenyl 4-vinylbenzene sulfonate (PFP-4-VBS) (22)

The reaction was carried out according to literature.[149]

First, 4-vinylbenzene-1-sulfonyl chloride was synthesized then the active ester was made in a second step:

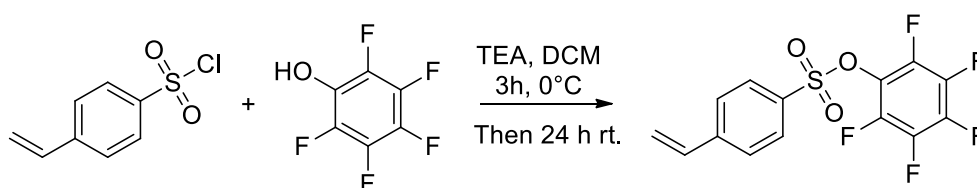


Under an argon atmosphere, sodium styrenesulfonate (21.8 mmol, 4.5 g) was added in small portions to thionyl chloride (109 mmol, 12.97 g, 8 ml) over 10 minutes at 0°C while stirring. To the resulting suspension, *N,N*-dimethylformamide (DMF) (11 ml) was added dropwise under cooling. The reaction mixture became homogeneous and was stirred for 3 hours at room temperature (RT). The reaction mixture was then poured into ice water to quench any unreacted thionyl chloride. The organic product was extracted with diethyl ether (2x). The ether layer was washed twice with 2% hydrochloric acid (HCl) solution and twice with water. The organic phase was dried over sodium sulfate (Na_2SO_4) and the solvent was removed under reduced pressure. The product, 4-vinylbenzene-1-sulfonyl chloride, was immediately frozen to prevent the hydrolysis of the sulfonyl chloride.

Yield: 75%, 3.3 g, 16.3 mmol as yellow liquid.

Spectral data:

^1H NMR (500 MHz, CDCl_3), δ ppm: δ 8.02 – 7.93 (m, 2H, Ar), 7.64 – 7.55 (m, 2H, Ar), 6.86 – 6.70 (m, 1H, $-\text{CH}=\text{}$), 5.95 (t, $J = 10.4$ Hz, 1H, $=\text{CH}_2$), 5.53 (d, $J = 10.9$ Hz, 1H, $=\text{CH}_2$).



To a solution of pentafluorophenol (1.7 g, 9.25 mmol) and triethylamine (1.32 ml, 0.96 g, 9.5 mmol) in 10 ml of dichloromethane (DCM), p-styrenesulfonyl chloride (1.76 g, 8.75 mmol) was added slowly at 0°C under a nitrogen atmosphere. The solution was then stirred for 3 hours at 0°C, followed by stirring for 24 hours at room temperature (RT). The reaction mixture was washed twice with water and twice with brine. The organic phase was dried over anhydrous magnesium sulfate (MgSO₄). After evaporation of the solvent and drying under vacuum, a light brown solid was obtained.

NMR analysis (¹⁹F and ¹H) indicated the formation of the product. Thin-layer chromatography (TLC) showed a clear difference between the spots, confirming the product formation (R_F: 0.8, DCM:HEX, 1:1).

Yield: 47%, 1.459 g, 4.27 mmol.

Melting point (MP): 53°C.

Spectral data:

Elemental analysis calculated: C 48.01%, H 2.01%, S 9.15%; found: C 48.05%, H 2.01%, S 9.14%.

¹H NMR (500 MHz, CDCl₃), δ ppm: 7.95 – 7.90 (m, 2H, Ar), 7.63 – 7.58 (m, 2H, Ar), 6.79 (dd, *J* = 17.6, 10.9 Hz, 1H, -CH=), 5.98 (dd, *J* = 17.6, 3.0 Hz, 1H, =CH₂), 5.54 (d, *J* = 10.9 Hz, 1H, =CH₂).

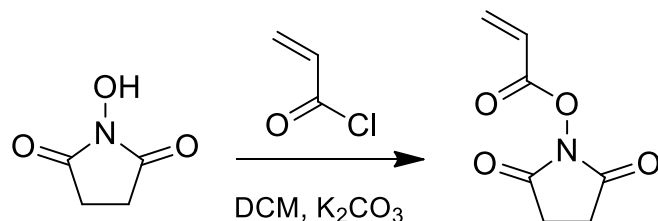
¹³C NMR (126 MHz, CDCl₃), δ ppm: 144.53 (s), 135.01 (s), 133.36 (s), 129.01 (s), 127.13 (s), 119.20 (s).

¹⁹F NMR (471 MHz, CDCl₃), δ ppm: -150.01 – -150.87 (m, 2F), -154.95 – -155.87 (m, 1F), -160.35 – -161.61 (m, 2F).

NMR spectra are found in Fig. SI 2, Fig. SI 3, Fig. SI 4 and Fig. SI 5 in appendix.

N-hydroxysuccinimide acrylate (NHSA) (**23**)

The reaction was carried out according to a modified procedure from literature.[148,150]



N-Hydroxysuccinimide (NHS) (2 g, 17.4 mmol) was dissolved in dichloromethane (DCM) (60 ml). Despite incomplete dissolution, the reaction was carried out in a heterogeneous mixture. Potassium carbonate (K_2CO_3) (2.4 g, 22 mmol) was added to the mixture and stirred at 0°C. Acryloyl chloride (1.8 ml, 17.4 mmol) was diluted in 20 ml DCM and added dropwise to the mixture. The reaction was allowed to proceed at 25°C overnight. The reaction mixture was washed three times with water and twice with brine. The organic phase was collected, and the solvent was evaporated to yield white crystals. After cooling to room temperature, the crystals were recrystallized from a Hexane/acetate (4:1) mixture. Pure white crystals were obtained by filtration.

Yield: 49%, 1.44 g, 8.5 mmol.

Spectral data:

^1H NMR (500 MHz, $CDCl_3$), δ ppm: 6.75 – 6.66 (m, 1H, -CH=), 6.40 – 6.25 (m, 1H, =CH₂), 6.23 – 6.10 (m, 1H, =CH₂), 2.86 (s, 4H).

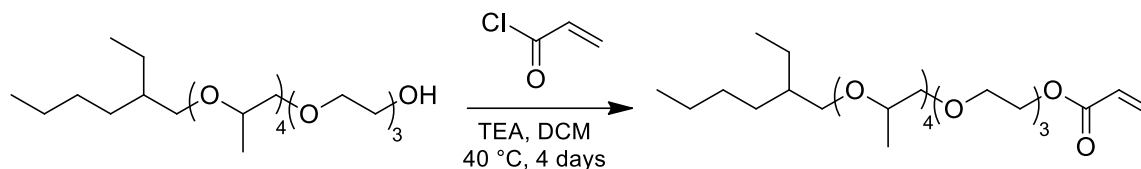
^{13}C NMR (126 MHz, $CDCl_3$), δ ppm: 169.46, 161.49, 136.62, 123.40, 26.05.

NMR spectra are found in Fig. SI 6 and Fig. SI 7 in appendix.

5.4 Synthesis of Surfactant and Biofilm Disruptor Monomers

Synthesis of Ecosurf-Surfactant acrylate EH3A (**24**)

The reaction was carried out according to a modified procedure from literature.[137]



In a three-neck round-bottom flask equipped with a gas inlet, dropping funnel, and reflux condenser, EH3 (27.8 g, 58.28 mmol) and triethylamine (TEA) (5.7 g, 56.3 mmol) were dissolved in 80 ml dichloromethane (DCM) and stirred for one hour under an argon atmosphere. Subsequently, acryloyl chloride (9.8 g, 108 mmol) diluted in 80 ml DCM was added dropwise slowly with vigorous stirring and ice cooling. The solution was stirred at 40°C for four days. For purification, the solvent was evaporated under reduced pressure. The residue was dissolved in 300 ml sodium hydroxide solution (1 M) and extracted with DCM (2 × 150). The organic phases were combined, dried over magnesium sulfate (MgSO₄), and the solvent was evaporated under reduced pressure. The residue was dissolved in 300 ml DCM and extracted with hydrochloric acid (1 M, 2 x 150 ml). The aqueous phase was extracted with 150 ml DCM, and the organic phases were combined, dried over magnesium sulfate, and the solvent was evaporated under reduced pressure. The resulting slightly yellow, viscous product was dried overnight under oil pump vacuum.

Yield: 80%, 24.74 mmol, 24.7 g (90 % pure).

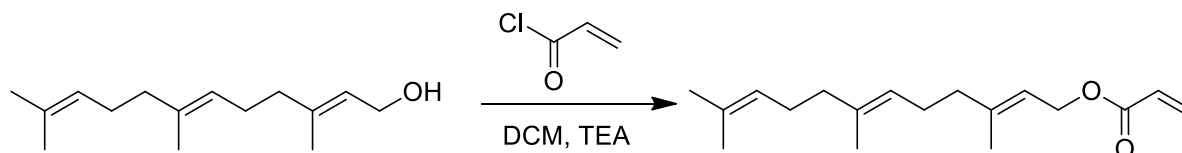
Spectral data:

¹H NMR (500 MHz, CDCl₃), δ ppm: 0.86 (t, 6H, 2x -CH₃), 1.14-1.27 (m, 20H, 4x CH₃ and 4x -CH₂-), 1.49 (m, 1H, -CH-), 3.29-3.74 (m, 22H, 4x -CH and 9x -CH₂-O-), 4.30 (m, 2H, -CH₂-O-), 5.81 (m, 1H, -CH=CH₂), 6.14 (m, 1H, -CH=CH₂), 6.41 (m, 1H, -CH=CH₂)

NMR spectra is found in Fig. SI 8 in appendix.

Synthesis of (2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl acrylate (Farnesol acrylate) (19)

The reaction was carried out according to a modified procedure from literature.[137]



Farnesol (1.00 g, 4.50 mmol) and triethylamine (1.25 ml, 911 mg, 9.00 mmol, 2 eq.) were dissolved in 20 ml of dichloromethane (DCM). The solution was cooled in an ice bath, and acryloyl chloride (0.732 ml, 815 mg, 9.00 mmol, 2 eq.) in 20 ml of DCM was added dropwise. The solution was stirred overnight. The solvent was removed under reduced pressure, resulting in a yellow oily solid. The solid was redissolved in 100 ml of DCM and washed with sodium hydroxide solution (1 M, 2 × 70 ml). The aqueous phases were combined and extracted with 40 ml of DCM. The organic phases were combined and washed with hydrochloric acid (1 M, 70 ml). The aqueous phase was extracted with 40 ml of DCM. The combined organic phases were washed with water (2 × 50 ml), dried over sodium sulfate (Na_2SO_4), and the solvent was removed under reduced pressure to yield the product as a yellow liquid.

Yield: 100%, 1.24 g, 4.49 mmol.

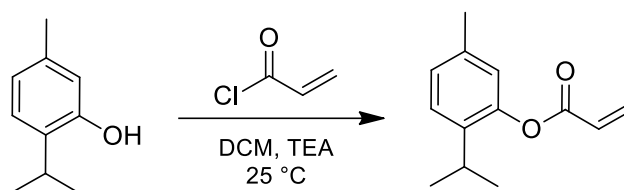
Spectral data:

^1H NMR (500 MHz, CDCl_3), δ ppm: 1.60 (s, 3H), 1.70 (m, 9H), 2.05 (m, 8H), 4.68 (d, $^3J = 7.2$ Hz, 2H), 5.10 (m, 2H), 5.38 (t, $^3J = 7.2$ Hz, 1H), 5.81 (dt, $^2J = 1.8$ Hz, $^3J = 10.4$ Hz, 1H), 6.13 (qt, $^3J = 10.4$ Hz, $^3J = 17.4$ Hz, 1H), 6.40 (dt, $^2J = 17.4$ Hz, $^3J = 1.4$ Hz, 1H).

NMR spectra is found in Fig. SI 9 in appendix.

Synthesis of 2-isopropyl-5-methylphenyl acrylate (Thymol acrylate) (20)

The reaction was carried out according to a modified procedure from literature.[151]



2-Isopropyl-5-methylphenol (700 mg, 4.66 mmol) and triethylamine (1.29 ml, 943 mg, 9.32 mmol, 2 eq.) were dissolved in 20 ml of dichloromethane (DCM). The solution was cooled in an ice bath, and acryloyl chloride (0.757 ml, 844 mg, 9.32 mmol, 2 eq.) in 20 ml of DCM was added dropwise. The solution was stirred overnight. The solvent was removed under reduced pressure, yielding a yellowish solid. The solid was redissolved in 50 ml of DCM and washed with sodium hydroxide solution (1 M, 2 × 70 ml). The aqueous phases were combined and extracted with 40 ml of DCM. The combined organic phases were washed with hydrochloric acid (1 M, 70 ml). The aqueous phase was extracted with 40 ml of DCM. The combined organic phases were dried over sodium sulfate (Na₂SO₄) and the solvent was removed under reduced pressure to yield the product as a yellow liquid.

Yield: 94%, 898 mg, 4.40 mmol.

Spectral data:

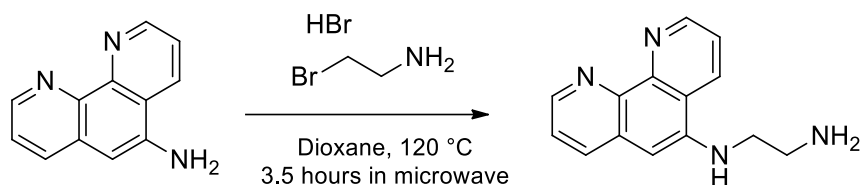
¹H NMR (500 MHz, CDCl₃), δ ppm: 1.19 (d, ³J = 6.9 Hz, 6H, -CH-CH₃), 2.32 (s, 3H, -CH₃), 2.98 (sep, ³J = 6.9 Hz, 1H, -CH-), 6.02 (dd, ²J = 1.2 Hz, ³J = 10.5 Hz, 1H), 6.35 (dd, ³J = 10.5 Hz, ³J = 17.3 Hz, 1H), 6.61 (dd, ²J = 1.2 Hz, ³J = 17.3 Hz, 1H), 6.86 (d, ⁴J = 1.0 Hz, 1H), 7.04 (d, ³J = 7.9 Hz, 1H), 7.21 (d, ³J = 7.9 Hz, 1H).

NMR spectra is found in Fig. SI 10 in appendix.

5.5 Synthesis of Phenanthroline Derivatives

Synthesis of *N*-(1,10-phenanthrolin-5-yl)ethane-1,2-diamine (**1**)

The reaction was carried out according to a modified procedure from literature.[119]



5-Amino-1,10-phenanthroline (100 mg, 0.512 mmol) and 2-bromoethylamine hydrobromide (114 mg, 0.563 mmol, 1.15 eq.) were dissolved in approximately 10 ml of dioxane. The reactants were stirred at room temperature (RT) for 15 minutes under an argon atmosphere. The reaction vessel was sealed and heated in a microwave at 120°C for 3.5 hours, resulting in the precipitation of a brown solid. The solvent was removed under reduced pressure, and the crude product was added to 4 ml of water. The pH of the solution was adjusted to 10-11 using sodium hydroxide (NaOH) solution. The solvent was then removed under vacuum, yielding a highly viscous red oil. The crude product was purified using column chromatography on alumina. Initially, unreacted 5-amino-1,10-phenanthroline was eluted using DCM:MeOH (9:1), *R_F* = 0.7, then the remaining product was then eluted using DCM:MeOH:NH₂ (magic). Thin-layer chromatography (TLC) indicated the presence of some unreacted starting material, necessitating a second column chromatography using the same eluents.

Yield: 82%, 100 mg.

Spectral data:

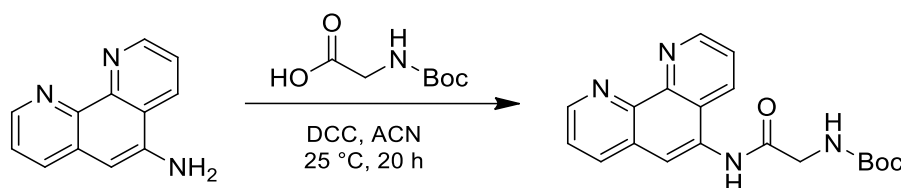
¹H NMR (500 MHz, DMSO-*d*₆), δ ppm: δ 9.02 (dd, *J* = 4.2, 1.4 Hz, 1H), 8.71 (ddd, *J* = 6.4, 5.8, 1.5 Hz, 2H), 8.06 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.66 (dd, *J* = 8.4, 4.2 Hz, 1H), 7.51 (dd, *J* = 8.1, 4.2 Hz, 1H), 6.70 (s, 1H, -NH-), 6.65 (t, *J* = 5.2 Hz, 1H), 3.50 (q, *J* = 5.8 Hz, 2H, -NH-CH₂-), 3.17 (t, *J* = 7.4, 4.0 Hz, 2H, -CH₂-NH₂).

ESI-MS analysis: a clear peak at 239 was detected (product + H) along with several peaks of the other fragments and isomers: at 222.24 (C₁₄H₁₂N₃-), 196.27 (C₁₂H₁₀N₃+) and 180.87 (C₁₂H₈N₂).

NMR spectra is found in Fig. SI 11 in appendix.

Synthesis of *tert*-butyl (2-((1,10-phenanthroline-5-yl)amino)-2-oxoethyl)carbamate (**2**)

The reaction was carried out according to literature.[120]



A solution of commercial *N*-(*tert*-butoxycarbonyl) glycine (1.31 g, 7.5 mmol) and DCC (0.8 g, 3.9 mmol) in acetonitrile (15 ml) was stirred at room temperature (RT) for 2 hours and filtered to remove the dicyclohexylurea precipitate. The resulting anhydride was used without further purification and added to a solution of 5-amino-1,10-phenanthroline (150 mg, 0.775 mmol) in 15 ml of acetonitrile. Although the second solution was initially poorly soluble, it dissolved completely upon addition of the anhydride solution. The mixture was stirred at RT for 20 hours under an argon atmosphere, and the solvent was then removed under reduced pressure. The remaining solid was dissolved in dichloromethane (DCM) and washed three times with water. The organic phase was filtered and dried to yield a yellow powder. The product was further purified by washing with a basic solution.

Yield of the crude product: 85%.

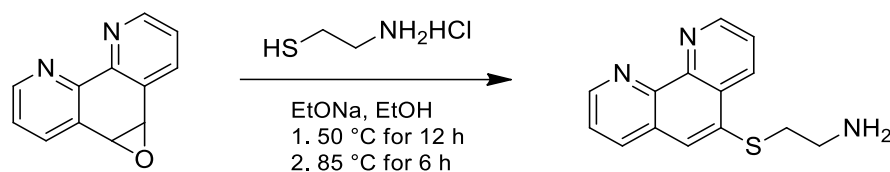
Spectral data:

^1H NMR (500 MHz, $\text{DMSO-}d_6$), δ ppm: δ 10.16 (s, 1H, Ar-NH-), 9.12 (dd, J = 4.2, 1.5 Hz, 1H), 9.04 (dd, J = 4.3, 1.7 Hz, 1H), 8.64 – 8.59 (m, 1H), 8.46 (dd, J = 8.1, 1.6 Hz, 1H), 8.13 (s, 1H), 7.80 (dd, J = 8.4, 4.3 Hz, 1H), 7.74 (dd, J = 8.1, 4.3 Hz, 1H), 7.21 (t, J = 5.8 Hz, 1H, -NH-Boc), 3.94 (d, J = 6.0 Hz, 2H, -CH₂-), 1.42 (d, J = 1.6 Hz, 9H, Boc group).

NMR spectra is found in Fig. SI 12 in appendix.

Synthesis of 2-((1,10-phenanthrolin-5-yl)thio)ethanamine (**3**)

The reaction was carried out according to a modified procedure from literature.[114]



Initially, 130 mg (1.147 mmol, 3 eq.) of 2-aminoethanethiol hydrochloride was dissolved in 27 mL of a sodium ethoxide solution in ethanol (0.1 M). This solution was then added to a separate solution containing 66 mg (0.337 mmol) of 5,6-epoxy-5,6-dihydro-[1,10]phenanthroline in 3 mL of dry ethanol, all at room temperature. The resulting reaction mixture was stirred under an argon atmosphere at 50°C for a duration of 15 hours. Subsequently, the mixture was heated to reflux (80°C, 5h) to facilitate the removal of water and to enhance aromaticity.

Upon cooling, a white solid precipitated. The solution displayed a pH of 6, and it was neutralized by the addition of a few drops of a saturated sodium bicarbonate (NaHCO₃) solution. This neutralization led to the more precipitation of the white solid material. The resulting solid was isolated by filtration, and the filtrate (solvent) was then evaporated. To the residue, 20 ml chloroform was added, and it was washed three times with 50 ml water. The organic phase was dried over sodium sulphate, filtered and the solvent was evaporated to yield a yellow viscous oil. After 48 h, some white material was accumulated, and this is probably small amounts of remaining salts which will not affect the next step, that's why a further purification was unnecessary.

Yield: 93%, 81 mg.

Spectral data:

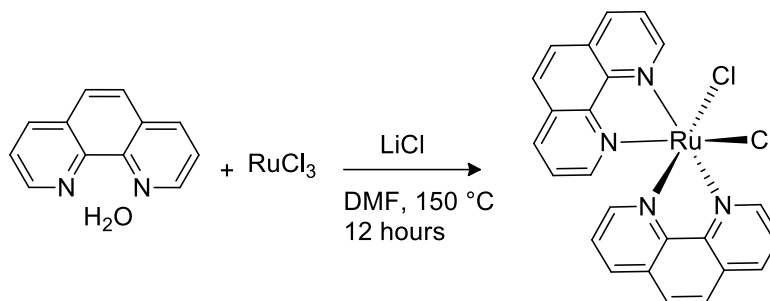
¹H NMR (500 MHz, CDCl₃), δ ppm: δ 9.21 (dd, *J* = 4.3, 1.6 Hz, 1H), 9.14 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.82 (dd, *J* = 8.3, 1.7 Hz, 1H), 8.16 (dd, 1H), 7.71 – 7.68 (m, 1H), 7.63 – 7.60 (m, 1H), 3.16 (t, 2H, -S-CH₂-), 3.00 (t, 2H, -CH₂-NH₂).

NMR spectra is found in Fig. SI 13 in appendix.

5.6 Synthesis of Ruthenium Complexes and Subsequent Monomers

Synthesis of $Ru(1,10\text{-Phenanthroline})_2Cl_2$ (**5**)

The reaction was carried out according to literature.[121]



$RuCl_3 \cdot 3H_2O$ (1 mmol, 261 mg, 1 eq.) and 1,10-phenanthroline monohydrate (397 mg, 2 eq.) were combined, and a solution of $LiCl$ (1.0 g) in 12 ml of dimethylformamide (DMF) was added to the mixture. The reaction was heated under reflux and then allowed to cool to room temperature (RT). The mixture was pipetted into 70 ml of water and centrifuged. The supernatant was decanted off, and the resulting dark solid was washed with 30 ml of water and centrifuged again. This washing and centrifugation step was repeated once more. The product was dried under vacuum at $60^{\circ}C$, yielding a dark-violet powder.

Yield: 43%, 245 mg.

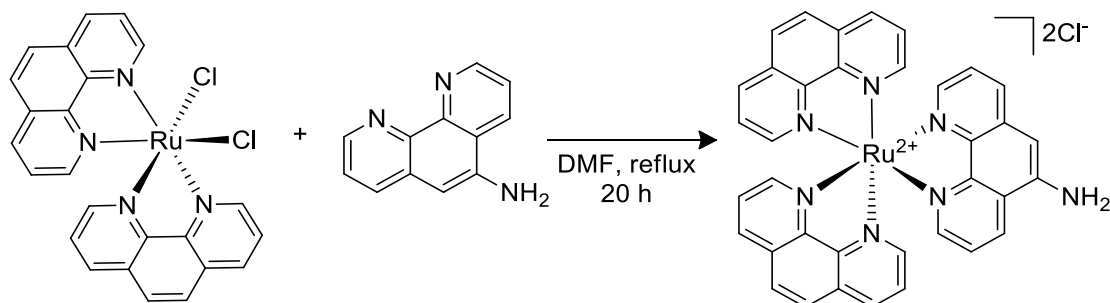
Spectral data:

^1H NMR (500 MHz, $DMSO-d_6$), δ (ppm) = 7.33 (dd, $^3J = 8.1$ Hz, $^4J = 5.5$ Hz, 2H), 7.75 (dd, $^3J = 5.5$ Hz, $^4J = 1.1$ Hz, 2H), 8.14 (d, $^3J = 8.9$ Hz, 2H), 8.23 (m, 4H), 8.29 (d, $^3J = 8.9$ Hz, 2H), 8.71 (dd, $^3J = 8.1$ Hz, $^4J = 1.2$ Hz, 2H), 10.28 (dd, $^3J = 5.2$ Hz, $^4J = 1.2$ Hz, 2H).

NMR spectra is found in Fig. SI 14 in appendix.

Synthesis of [Ru(1,10-phenanthroline)₂(1,10-phenanthrolin-5-amine)] Cl₂

The reaction was carried out according to a modified procedure from literature.[77,115,116]



In a 25 mL two-necked flask under argon, 63 mg of 5-amino-1,10-phenanthroline (0.32 mmol, 1.2 eq.) were added to 145 mg of **5** [Ru(phen)₂Cl₂] (0.268 mmol) dissolved in 4 mL of DMF. The mixture was heated at reflux overnight. The product didn't precipitate so the solvent was concentrated under reduced pressure and ethanol was added. An orange-reddish solid precipitated and was filtered. The solid was dissolved in DCM washed with water (3x) to yield a dark red powder.

Yield: 48.5%, 95 mg, 0.13 mmol.

Spectral data:

¹H NMR (500 MHz, Aceton-*d*₆), δ ppm: 8.92 (dd, 1H, *J* = 8.5, 1.1 Hz), 8.81 – 8.75 (m, 4H, Ar), 8.44 – 8.33 (m, 10H, Ar), 7.91 (dd, 1H, *J* = 5.1, 1.2 Hz, Ar), 7.87 – 7.73 (m, 6H, Ar), 7.52 (dd, 1H, *J* = 8.4, 5.1 Hz, Ar), 7.31 (d, 1H, *J* = 3.2 Hz, Ar), 6.41 (s, -NH₂).

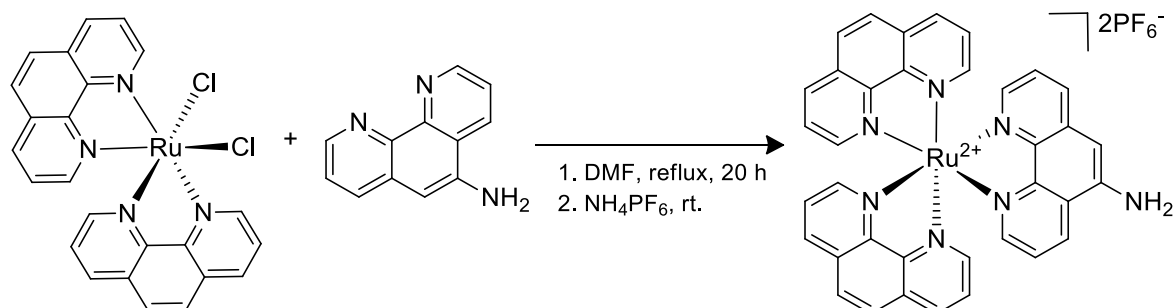
UV-Vis abs.: λ 350 nm – 500 nm, λ_{max} = 460 nm.

Phosphorescence: λ 550 nm – 750 nm, λ_{max} = 590 nm.

NMR spectra is found in Fig. SI 15 in appendix.

Synthesis of [Ru(1,10-phenanthroline)₂(1,10-phenanthrolin-5-amine)](PF₆)₂

The reaction was carried out according to a modified procedure from literature.[77,115,116]



In a 25 ml two-necked flask under an argon atmosphere, 24 mg of 5-amino-1,10-phenanthroline (0.12 mmol, 1.2 eq.) were added to 52 mg of **5** (0.1 mmol) dissolved in 4 ml of dimethylformamide (DMF). The mixture was heated at reflux overnight. After cooling to room temperature (RT), a saturated aqueous solution of NH₄PF₆ was added. Since the product did not precipitate, the solvent was concentrated under reduced pressure, and ethanol was added, resulting in the precipitation of an orange-reddish solid. The solid was filtered, washed with ethanol and water, and dried to yield a red powder.

Yield: 53%, 52 mg, 0.053 mmol.

The experiment was also performed using ethanol as the solvent, and the yield was similar.

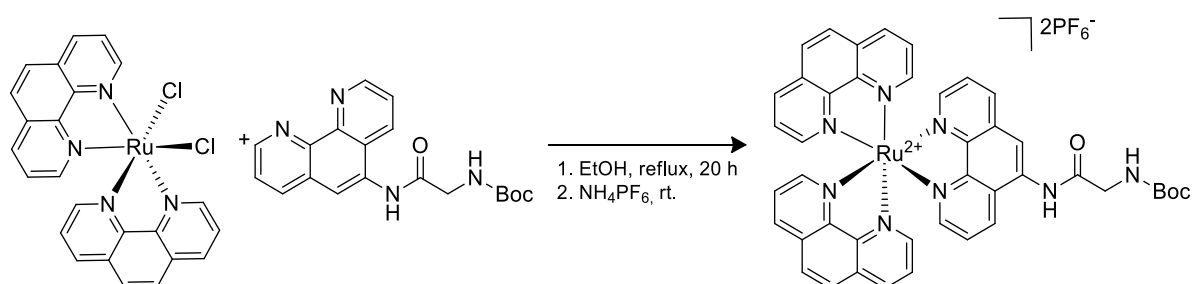
Spectral data:

¹H NMR (500 MHz, Aceton-*d*₆), δ (ppm) = δ 8.94 – 8.88 (d, 1H), 8.81 – 8.74 (m, 4H), 8.43 – 8.29 (m, 10H), 7.92 – 7.71 (m, 6H), 7.52 (d, 1H), 7.30 (s, 1H), 6.42-4.38 (s).

NMR spectra is found in Fig. SI 16 in appendix.

Synthesis of $[Ru(1,10\text{-phenanthroline})_2(\text{tert-butyl } (2\text{-}((1,10\text{-phenanthrolin-5-yl})\text{amino})\text{-2-oxoethyl})\text{carbamate})] (PF_6)_2$ (7**)**

The reaction was carried out according to a modified procedure from literature.[77,115,116]



In a 50 mL three-necked flask under argon, *tert*-butyl (2-((1,10-phenanthrolin-5-yl)amino)-2-oxoethyl)carbamate ([62.5 mg, 80% purity], 50 mg, 0.142 mmol, 1.2 eq.) were added to 67 mg of **5** (0.118 mmol) dissolved in 18 mL of Ethanol. The mixture was heated at reflux overnight. Saturated aqueous solution of NH_4PF_6 was added to the solution at room temperature. The resulting precipitate was collected by filtration, dissolved in DCM, and washed three times with water. A dark red powder was obtained after drying the organic phase.

Yield: 61%, 80 mg, 0.072 mmol.

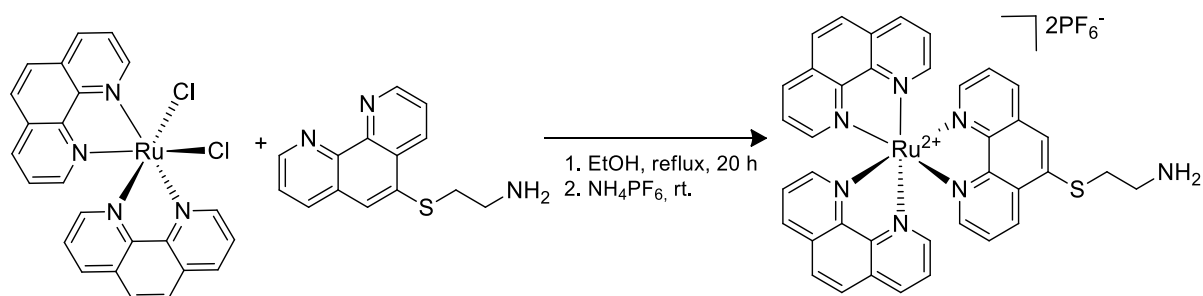
Spectral data:

^1H NMR (500 MHz, Aceton- d_6), δ ppm: δ 10.39 – 10.22 (m, 1H, -NH-Boc), 9.07 (t, J = 10.3 Hz, 1H, Ar), 8.78 (ddt, J = 4.0, 2.3, 1.2 Hz, 4H, Ar), 8.67 (dd, J = 20.9, 7.5 Hz, 2H, Ar), 8.44 – 8.36 (m, 9H, Ar), 8.28 (dd, J = 5.2, 1.0 Hz, 1H, Ar), 7.82 – 7.75 (m, 5H, Ar), 7.72 (dd, J = 8.3, 5.3 Hz, 1H, Ar), 6.66 (d, J = 35.8 Hz, 1H, Ar-NH-), 4.14 (d, J = 5.2 Hz, 2H, -CH₂-), 1.44 (s, 9H, -Boc).

NMR spectra is found in Fig. SI 17 in appendix.

Synthesis of $[Ru(1,10\text{-phenanthroline})_2(2\text{-}((1,10\text{-phenanthrolin-5-yl})\text{thio})\text{ethanamine})](PF_6)_2$ (8**)**

The reaction was carried out according to a modified procedure from literature.[77,115,116]



In a 50 mL three-necked flask under argon, 30 mg of 2-((1,10-phenanthrolin-5-yl)thio)ethanamine (0.058 mmol) were added to 32 mg of **5** (0.058 mmol) dissolved in 6 mL of Ethanol. The mixture was heated at reflux overnight. Saturated aqueous solution of NH_4PF_6 was added to the solution at room temperature. The resulting precipitate was collected by filtration, dissolved in DCM, and washed three times with water. A dark red powder was obtained after drying the organic phase.

Yield: 55%, 32 mg, 0.032 mmol.

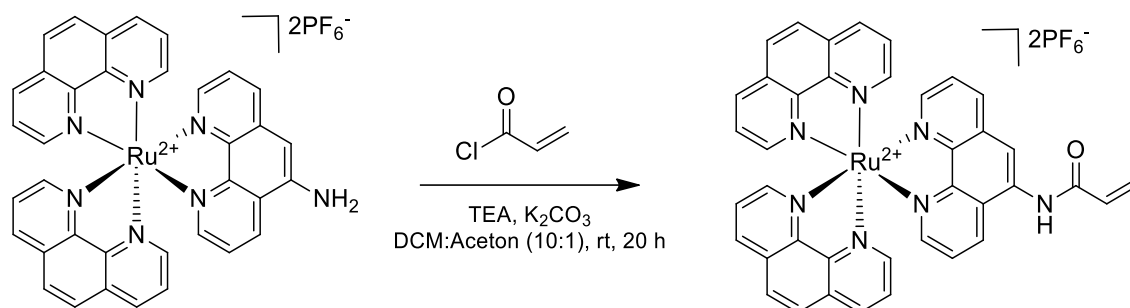
Spectral data:

^1H NMR (500 MHz, Aceton- d_6), δ ppm: δ 8.93 – 8.90 (m, 1H, Ar), 8.81 – 8.77 (m, 6H, Ar), 8.68 – 8.65 (m, 1H, Ar), 8.44 – 8.36 (m, 9H, Ar), 8.28 (dd, $J = 5.2, 1.2$ Hz, 1H, Ar), 7.84 – 7.77 (m, 7H, Ar), 7.74 (dd, $J = 8.3, 5.2$ Hz, 1H, Ar), 3.66 (t, $J = 6.5$ Hz, 2H, -S-CH₂-), 3.61 – 3.57 (t, 2H, -CH₂-NH₂).

NMR spectra is found in Fig. SI 18 in appendix.

Synthesis of $[Ru(1,10\text{-phenanthroline})_2 (N\text{-(1,10-phenanthrolin-5-yl) acrylamide})] (PF_6)_2$ (11**)**

The reaction was carried out following a general procedure to make monomers using acryloyl chloride.[137]



40 mg (0.05 mmol) of $[Ru(1,10\text{-phenanthroline})_2 (1,10\text{-phenanthrolin-5-amine})] (PF_6)_2$ was added to 10 ml of dichloromethane (DCM), in which it was poorly soluble. Triethylamine (TEA, 10 mg, 0.1 mmol) was then added, followed by the gradual dropwise addition of acryloyl chloride (9 mg, 0.1 mmol) diluted in DCM. The mixture was stirred at room temperature (RT) for 3 hours, after which a few drops of acetone were added to enhance solubility, resulting in complete dissolution. TLC monitoring showed no change in the spots, so potassium carbonate (K_2CO_3 , 24 mg, 0.18 mmol) was added as an inorganic base. To ensure full conversion of the reaction, an additional 9 mg of acryloyl chloride was added dropwise. After 20 hours, the solvents were evaporated, and the residue was dissolved in DCM. The organic phase was washed three times with water, dried over magnesium sulfate ($MgSO_4$), filtered, and evaporated to obtain the product as a red powder.

Yield: 90%, 39 mg, 0.045 mmol.

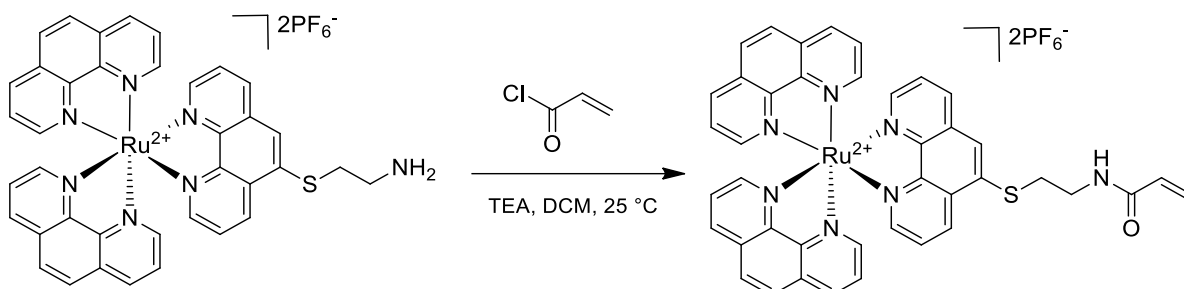
Spectral data:

^1H -NMR (500 MHz, Aceton- d_6), δ ppm: 9.96 (s, 1H, -NH-), 9.03 – 8.94 (m, 1H, Ar), 8.87 (s, 1H, Ar), 8.84 – 8.76 (m, 6H, Ar), 8.74 – 8.68 (m, 1H, Ar), 8.46 – 8.33 (m, 13H, Ar), 8.33 – 8.26 (m, 1H, Ar), 7.87 – 7.72 (m, 9H, Ar), 7.69 – 7.61 (m, 1H, Ar), 6.83 – 6.71 (m, 1H, -CH=), 6.55 – 6.47 (m, 1H, =CH₂), 5.90 (dd, 1H, J = 10.2, 1.8 Hz, =CH₂).

NMR spectra is found in Fig. SI 19 in appendix.

*Synthesis of [Ru(1,10-phenanthroline)₂ (N-(2-((1,10-phenanthrolin-5-yl)thio) ethyl) acrylamide)] (PF₆)₂ (**12**)*

The reaction was carried out following a general procedure to make monomers using acryloyl chloride.[137]



8 (55 mg, 0.055 mmol) was dissolved in DCM (15 ml) and triethylamine (TEA, 10 μ l, 7.3 mg, 0.072 mmol, 1.3 eq.) was added. While cooling with a water bath, acryloyl chloride (9.0 μ l, 10 mg, 0.11 mmol, 2 eq.) in DCM (2-3 ml) was added dropwise. The reaction mixture was then stirred for 22 hours. The solvent was removed under reduced pressure, and the residue was redissolved in DCM (25 ml) and washed with water (3 $\times$ 30 ml). The aqueous phase was extracted with DCM (2 $\times$ 25 ml). The combined organic phases were dried over sodium sulfate (Na₂SO₄) and the solvent was removed under reduced pressure to yield a dark red solid (still around 25% non-acrylate). Purification by column chromatography (neutral aluminum oxide 90, DCM $\rightarrow$ DCM:EtOH, 9:1) yielded a dark red solid.

Yield: 58%, 34 mg, 0.032 mmol.

Spectral data:

¹H NMR (500 MHz, Aceton-*d*₆), δ ppm: 8.87 (dd, *J* = 8.6 Hz, *J* = 1.2 Hz, 1H), 8.79 (m, 5H), 8.71 (dd, *J* = 8.4 Hz, *J* = 1.2 Hz, 1H), 8.69 (s, 1H), 8.41 (m, 11H), 8.29 (dd, *J* = 5.3 Hz, *J* = 1.2 Hz, 1H), 7.81 (m, 6H), 7.75 (dd, *J* = 8.4 Hz, *J* = 5.3 Hz, 1H), 6.27 (dd, ²*J* = 17.1 Hz, ³*J* = 10.0 Hz, 1H, =CH₂), 6.19 (dd, ²*J* = 17.1 Hz, ³*J* = 2.4 Hz, 1H, =CH₂), 5.55 (dd, ²*J* = 10.0 Hz, ³*J* = 2.4 Hz, 1H, -CH=), 3.67 (m, 2H, -CH₂N-), 3.52 (t, ³*J* = 7.3 Hz, 2H, -SCH₂-).

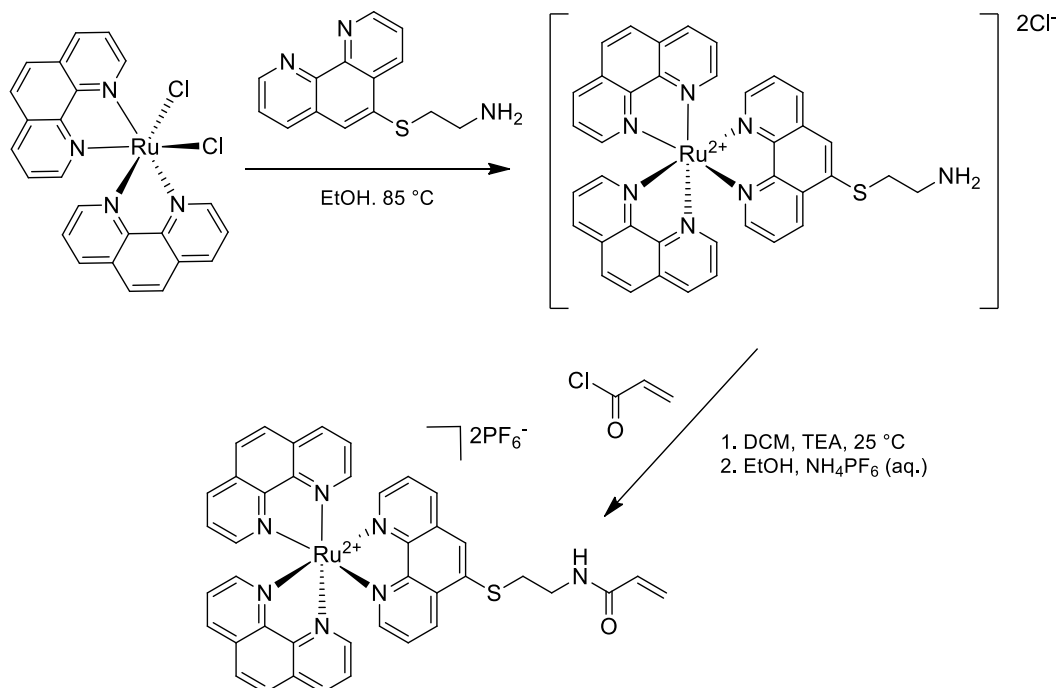
¹³C NMR (126 MHz, Aceton-*d*₆), δ ppm: 165.5, 153.39, 153.32, 153.17, 152.18, 148.35, 148.11, 146.88, 137.08, 136.39, 135.70, 133.45, 131.42, 131.17, 131.06, 130.18, 128.29, 126.64, 126.27, 126.12, 125.24, 38.1, 31.35.

UV-Vis abs.: λ 350 nm – 500 nm, λ_{max} : 450 nm.

^1H NMR spectra is found in Figure 13 in Results and Discussion chapter.

^{13}C NMR spectra is found in Fig. SI 20 in appendix.

One pot synthesis of $[Ru(1,10\text{-phenanthroline})_2(N\text{-}(2\text{-}((1,10\text{-phenanthrolin-5-yl)thio)ethyl)acrylamide)](PF_6)_2$ (**12**)



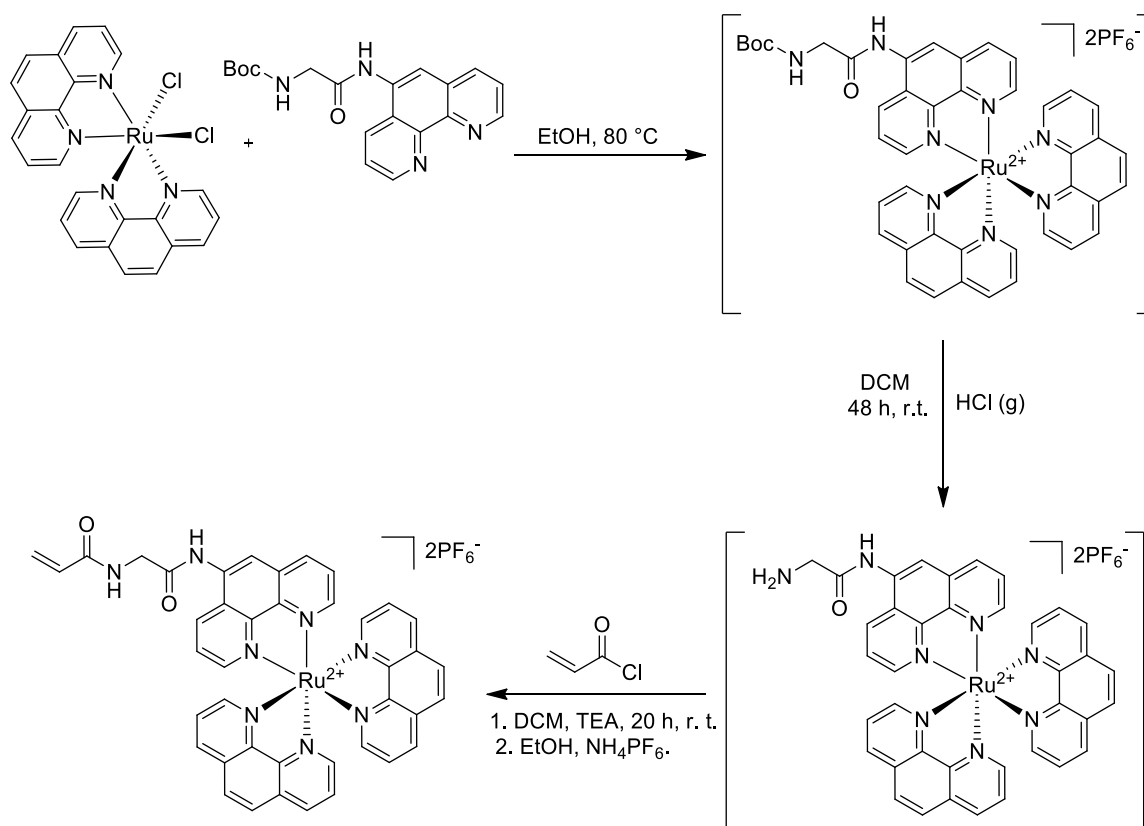
3 (179 mg, 0.701 mmol, 1.2 eq.) and **5** (311 mg, 0.584 mmol) were dissolved in dry ethanol (50 ml) and heated under argon atmosphere at reflux overnight. After 20 h, the reaction was cooled down and the solvent was removed under reduced pressure. The crude product was used without further purification.

The obtained product (460 mg, 0.584 mmol) was dissolved in DCM (60 ml) and triethylamine (TEA, 325 μ l, 237 mg, 2.34 mmol, 4 eq.) was added. While cooling with a water bath, acryloyl chloride (190 μ l, 212 mg, 2.34 mmol, 4 eq.) in DCM (20 ml) was added dropwise. The reaction mixture was then stirred overnight. The solvent was removed under reduced pressure, and residual triethylamine and acryloyl chloride were removed under oil pump vacuum. The solid residue was redissolved in ethanol, and a saturated ammonium hexafluorophosphate solution (12 drops) was added dropwise. The precipitated solid was separated *via* centrifugation, washed with ethanol, centrifuged again, and dried under oil pump vacuum for 3 hours. The product was obtained as an orange powder.

Yield: 44% (over all steps), 274 mg, 0.258 mmol.

The obtained spectral data were similar to the previous experiment.

One pot synthesis of $[Ru(1,10\text{-phenanthroline})_2(N\text{-}(2\text{-}((1,10\text{-phenanthrolin-5-yl})\text{amino})\text{-2-oxoethyl})\text{acrylamide})](PF_6)_2$ (**13**)



2 (100 mg, 0.284 mmol, 1.2 eq.) and **5** (126 mg, 0.237 mmol) were dissolved in dry ethanol (40 mL) and heated under argon atmosphere at reflux overnight. After cooling, the solvent was removed under reduced pressure and used without purification for the next step.

The crude product (210 mg, 0.236 mmol) was dissolved in DCM (20 ml). The mixture was stirred under HCl gas at room temperature for 2 days, with occasional addition of more HCl gas. After two days, a dark red solid formed at the bottom of the flask. The DCM was decanted, and the flask was washed once more with DCM (10 ml). The product was used without further purification.

This product (186 mg, 0.237 mmol) was dissolved in DCM (35 ml) and triethylamine (TEA, 198 μ l, 144 mg, 1.43 mmol, 5 eq.) was added. While cooling with a water bath, acryloyl chloride (115 μ l, 129 mg, 1.43 mmol, 5 eq.) in DCM (15 ml) was added dropwise. The reaction mixture was then stirred overnight. Since undissolved reactant was still visible the next day, it was removed from the mixture and dissolved in a separate flask in DCM (25 ml) and triethylamine (99 μ l, 72 mg, 0.72 mmol). Acryloyl

chloride (58 μ l, 65 mg, 0.72 mmol) in DCM (15 ml) was then added dropwise. The reaction was stirred overnight, both flasks were combined, and the solvent was removed under reduced pressure. To remove any remaining triethylamine or acryloyl chloride, the product was dried for a few hours under oil pump vacuum. The orange solid was dissolved in ethanol (15-20 ml), and the product was precipitated using a saturated ammonium hexafluorophosphate solution (approximately 15 drops). The precipitate was separated *via* centrifugation. The supernatant was decanted, and the solid was washed once with ethanol (15 ml) and centrifuged again. Drying under oil pump vacuum yielded an orange powder.

Yield: 52% (over all steps), 131 mg, 0.124 mmol.

Spectral data:

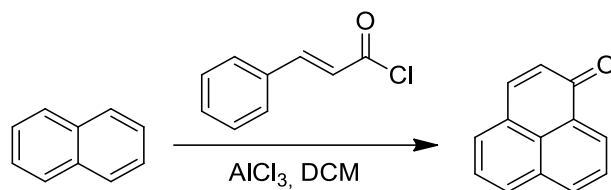
^1H NMR (500 MHz, Aceton- d_6), δ ppm: 10.23 (s, 1H, NH-Phen), 8.97 (d, J = 8.5 Hz, 1H, H-Ar), 8.79 (m, 6H, H-Ar), 8.75 (d, J = 6.5 Hz, 1H, H-Ar), 8.70 (d, J = 8.5 Hz, 1H, H-Ar), 8.41 (m, 14H, H-Ar), 8.29 (dd, J = 5.2 Hz, J = 1.0 Hz, 1H, H-Ar), 8.15 (br s, 1H, -NH-Acryl), 7.81 (m, 8H, H-Ar), 7.74 (dd, J = 8.3 Hz, J = 5.2 Hz, 1H, H-Ar), 6.63 (s, 1H, NH-acrylate), 6.44 (dd, ^2J = 17.0 Hz, ^3J = 10.2 Hz, 1H, -CH=), 6.31 (dd, ^2J = 17.0 Hz, ^3J = 2.0 Hz, 1H, =CH₂), 5.70 (dd, ^2J = 10.2 Hz, ^3J = 2.0 Hz, 1H, =CH₂), 4.31 (m, 2H, -CH₂NH-).

^{13}C NMR (126 MHz, Aceton- d_6), δ ppm: 169.20, 166.40, 153.34, 153.30, 153.29, 153.20, 153.17, 152.06, 148.51, 148.14, 148.10, 145.84, 137.08, 136.42, 134.15, 132.27, 131.17, 130.91, 128.34, 126.67, 126.27, 125.72, 44.46.

NMR spectra are found in Fig. SI 21 & Fig. SI 22 in appendix.

5.7 Synthesis of Phenalene-1-one and Subsequent Monomer

Synthesis of 1H-phenalene-1-one (**14**)



Method A (adapted from Godard et al.)[78]

In 4 microwave glasses, each containing naphthalene (0.96 g, 7.5 mmol) and cinnamoyl chloride (1.25 g, 7.5 mmol), DCM (8 ml) was added, and the mixtures were stirred in an ice bath for 10 min. Anhydrous AlCl_3 (3.00 g, 22.5 mmol) was added slowly, and the mixtures were stirred for 10 min at around 4 °C. The mixtures were irradiated in the microwave (100 W, 40 psi, 50 °C) for 12 min and then slowly poured into hydrochloric acid (37%, 400 ml). The precipitated solids were filtered off and washed with DCM. The filtrate was diluted with water (150 ml) and extracted with DCM (3 × 150 ml). The combined organic phases were washed with water (150 ml), dried over sodium sulphate, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (SiO_2 , hexane:EtOAc, 5:1, Rf: 0.46). The product was obtained as a yellow-orange solid.

Yield: 57%, 3.08 g, 17.1 mmol.

Spectral data:

^1H NMR (500 MHz, CDCl_3), δ ppm: 6.74 (d, J = 9.8 Hz, 1H), 7.60 (dd, J = 8.3 Hz, J = 7.1 Hz, 1H), 7.78 (m, 3H), 8.03 (d, J = 8.3 Hz, 1H), 8.21 (dd, J = 8.1 Hz, J = 1.1 Hz, 1H), 8.64 (dd, J = 7.4 Hz, J = 1.2 Hz, 1H).

NMR spectra is found in Fig. SI 23 in appendix.

Alternative synthetic routes were explored:

Method B (adapted from Xu et al.)[122,123]

Room temperature reaction of naphthalene and cinnamoyl chloride with AlCl_3 in DCM. The desired product was not obtained after attempted purification.

Method C (adapted from Song et al.)[123]

Reaction of naphthalene and cinnamoyl chloride with AlCl_3 in DCM at 40 °C.

Yield: 10%, 0.72 g, 4.0 mmol.

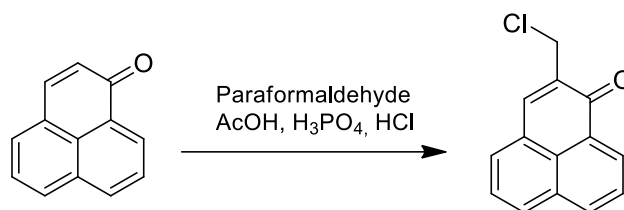
Method D (adapted from Späth et al., originally based on Fieser and Hershberg)[124,125]

Reaction of 2-naphthol with glycerine and sodium-3-nitrobenzenesulfonate in $\text{H}_2\text{SO}_4/\text{H}_2\text{O}$ at 140 °C.

Yield: 30%, 1.97 g, 10.9 mmol.

These alternative methods resulted in lower yields or unsuccessful synthesis and were not pursued further.

Synthesis of 2-(chloromethyl)-1*H*-phenalen-1-one (**15**)



Method A (adapted from Godard et al.)[78]

In a 100 mL round-bottom flask, phenalen-1-one (865 mg, 4.8 mmol) and paraformaldehyde (2.64 g, 12 mmol) were dissolved in glacial acetic acid (19 mL) and phosphoric acid (85%, 12 mL). The mixture was stirred at 110 °C until complete solubilization. Hydrochloric acid (37%, 13 mL) was then added, and the solution was stirred for 3 days at 110 °C.

After cooling the solution to room temperature, it was poured into a mixture of ice and water (75 mL) and neutralized with sodium hydroxide solution (5 M). The resulting solution, which contained dark particles, was filtered and washed with sodium bicarbonate solution (1 M, 50 mL). The residue on the filter paper was dissolved in DCM (200 mL), filtered, and washed with water (150 mL). The organic phase was dried over sodium sulfate, and the solvent was removed under reduced pressure. The crude black product was purified by column chromatography (SiO₂, DCM:Hexane (3:1), R_f: 0.42), yielding a yellow solid.

Yield: 18.8 mg, 17.1 mmol, 2%.

Method B (adapted from Späth et al.)[124]

In a separate round-bottom flask, phenalen-1-one (865 mg) and paraformaldehyde (1.75 g) were combined in glacial acetic acid (4.2 mL) and phosphoric acid (85%, 2.6 mL). The mixture was stirred at 110 °C until complete solubilization. Hydrochloric acid (37%, 3.2 mL) was added gradually over a course of 15 minutes, and the solution was stirred for an additional 16 hours at 110 °C.

After cooling the solution to room temperature, it was poured into water (8 mL) and neutralized with sodium hydroxide solution (5 M). The resulting solution containing dark solids was extracted with DCM (4 × 22 mL), and the combined organic phases were washed with water (20 mL), dried over sodium sulfate, and the solvent was

removed under reduced pressure. The crude black product was purified by column chromatography (SiO₂, DCM; Rf: 0.78), yielding a yellow solid.

Yield: 26%, 569 mg, 2.49 mmol.

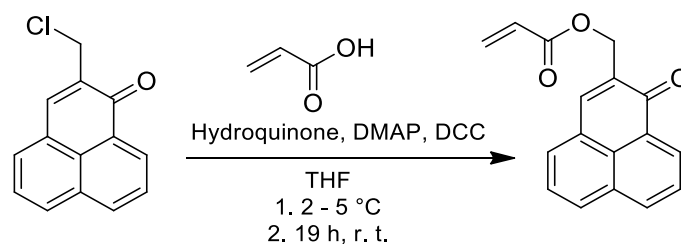
Spectral data:

¹H NMR (500 MHz, CDCl₃), δ ppm: 4.69 (d, *J* = 1.0 Hz, 2H), 7.63 (dd, *J* = 8.3 Hz, *J* = 7.1 Hz, 1H), 7.81 (m, 2H), 7.95 (s, 1H), 8.06 (d, *J* = 8.3 Hz, 1H), 8.23 (dd, *J* = 8.1 Hz, *J* = 1.0 Hz, 1H), 8.69 (dd, *J* = 7.4 Hz, *J* = 1.2 Hz, 1H).

¹³C NMR (126 MHz, CDCl₃), δ ppm: δ 183.59 (s), 140.39 (s), 135.74 (s), 135.26 (s), 132.30 (s), 132.10 (s), 131.02 (s), 129.10 (s), 127.40 (s), 127.33 (d, *J* = 10.3 Hz), 126.90 (s), 41.49 (s).

NMR spectra are found in Fig. SI 24 & Fig. SI 25 in appendix.

Synthesis of (1-oxo-1H-phenalen-2-yl)methyl acrylate (**16**)



Method A (adapted from Späth et al.)[126]

In a dry 50 mL round-bottom flask, 2-(Chloromethyl)-1H-phenalen-1-one (183 mg, 0.80 mmol) was dissolved in dry tetrahydrofuran (THF) (4 mL). To this solution, acrylic acid (183 μ L, 192 mg, 2.66 mmol, 3.33 equivalents), hydroquinone (28 mg, 0.26 mmol, 0.33 equivalents), DMAP (162 mg, 1.33 mmol, 1.66 equivalents), and DCC (274 mg, 1.33 mmol, 1.66 equivalents) were added while maintaining the temperature between 2-5 $^{\circ}$ C. The mixture was then stirred at room temperature overnight.

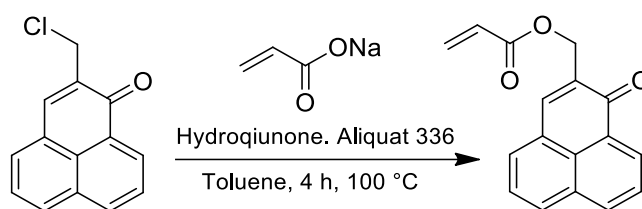
After the reaction period, ethyl acetate (55 mL) was added to the cooled solution and washed with water (80 mL). The aqueous phase was extracted with ethyl acetate (2 $\times$ 50 mL). The organic phases were combined, washed with water (100 mL), dried over sodium sulfate, and the solvent was removed under reduced pressure. The crude product was purified *via* column chromatography (SiO_2 , DCM, Rf: 0.89), yielding a bright yellow solid.

Yield: 185 mg (25% acrylate, 0.17 mmol, 21%).

Spectral data:

$^1\text{H-NMR}$ (500 MHz, CDCl_3), δ ppm: 4.69 (d, J = 1.0 Hz, 2H, $-\text{CH}_2-$), 6.02 (dd, ^2J = 1.2 Hz, ^3J = 10.5 Hz, 1H, $=\text{CH}_2$), 6.32 (dd, $3J$ = 10.4 Hz, ^3J = 17.3 Hz, 1H, $-\text{CH}=\text{)$, 6.61 (dd, ^2J = 1.2 Hz, ^3J = 17.3 Hz, 1H, $=\text{CH}_2$), 7.63 (dd, J = 8.3 Hz, J = 7.1 Hz, 1H, Ar-H), 7.81 (m, 2H, Ar-H), 7.95 (s, 1H, Ar-H), 8.05 (d, J = 8.3 Hz, 1H, Ar-H), 8.23 (dd, J = 8.1 Hz, J = 1.0 Hz, 1H, Ar-H), and 8.68 (dd, J = 7.4 Hz, J = 1.2 Hz, 1H, Ar-H).

Method B (adapted from Späth et al.)[126]



In a separate round-bottom flask, a solution of 2-(Chloromethyl)-1H-phenalen-1-one (430 mg, 1.88 mmol) was prepared in toluene (14 mL). Sodium acrylate (354 mg, 3.76 mmol, 2 equivalents), hydroquinone (41 mg, 0.38 mmol, 0.2 equivalents), and Aliquat®336 (750 mg) were added to the solution before stirring at reflux for four hours.

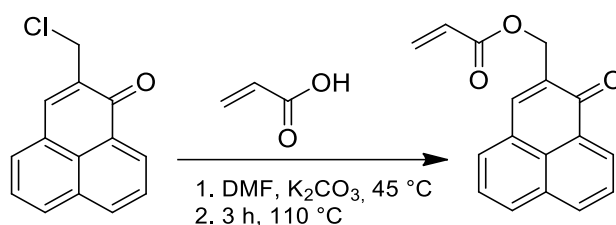
Following the reaction period and cooling down of the solution to room temperature, it was diluted with toluene (75 mL) and washed with water (120 mL). The aqueous phase was extracted with ethyl acetate (2 × 80 mL). The organic phases were combined and washed with water (120 mL), dried over sodium sulfate before removal of the solvent under reduced pressure. The crude product was purified *via* column chromatography (SiO₂, DCM; Rf:0.18), yielding a brown solid.

Yield: 242 mg (30% Aliquat®336), 0.61 mmol, 32%.

Spectral data:

¹H-NMR (500 MHz, CDCl₃), δ ppm: 5.31 (d, *J* = 1.2 Hz, 2H, -CH₂-), 5.89 (dd, ²*J* = 1.4 Hz, ³*J* = 10.5 Hz, 1H, =CH₂), 6.25 (dd, ³*J* = 10.5 Hz, ³*J* = 17.4 Hz, 1H, -CH=), 6.51 (dd, ²*J* = 1.4 Hz, ³*J* = 17.4 Hz, 1H, =CH₂), 7.61 (dd, *J* = 8.3 Hz, *J* = 7.1 Hz, 1H, Ar-H), 7.80 (m, 3H, Ar-H), 8.04 (d, *J* = 8.3 Hz, 1H, Ar-H), 8.22 (dd, *J* = 8.1 Hz, *J* = 1.0 Hz, 1H, Ar-H), 8.67 (dd, *J* = 7.4 Hz, *J* = 1.2 Hz, 1H, Ar-H).

Method C (adapted from Pittelkow et al.)[127]



Acrylic acid (0.31 mL, 0.32 g, 4.5 mmol, 2 equivalents) was dissolved in dimethylformamide (DMF) (5 mL) and potassium carbonate (0.68 g, 4.9 mmol, 2.2 equivalents) was added slowly to the solution while stirring at room temperature. Subsequently, a solution of 2-(Chloromethyl)-1H-phenalen-1-one (0.51 g, 2.2 mmol) in

DMF (7.5mL) was added to the mixture which was then heated to 100 °C for 3 hours under an argon atmosphere.

After cooling down, water (100 mL) was added to the mixture, followed by extraction with ethyl acetate (3×100mL). The organic phases were combined, dried over sodium sulphate, and the solvent was removed under reduced pressure. The product was then purified *via* column chromatography (SiO₂, DCM, Rf: 0.49) resulting in crystallization as a yellow-orange solid.

Yield: 65%, 377 mg, 1.43mmol.

Spectral data:

¹H NMR (500 MHz, CDCl₃), δ ppm: 4.69 (d, *J* = 1.0 Hz, 2H, -CH₂-), 6.02 (dd, ²*J* = 1.2Hz, ³*J* = 10.5 Hz, 1H, =CH₂), 6.32 (dd, ³*J* = 10.4 Hz, ³*J* = 17.3 Hz, 1H, -CH=), 6.61 (dd, ²*J* = 1.2 Hz, ³*J* = 17.3 Hz, 1H, =CH₂), 7.63 (dd, *J* = 8.3 Hz, *J* = 7.1 Hz, 1H, Ar-H), 7.81 (m, 2H, Ar-H), 7.95 (s, 1H, Ar-H), 8.05 (d, *J* = 8.3 Hz, 1H, Ar-H), 8.23 (dd, *J* = 8.1 Hz, *J* = 1.0 Hz, 1H, Ar-H), 8.68 (dd, *J* = 7.4 Hz, *J* = 1.2 Hz, 1H, Ar-H).

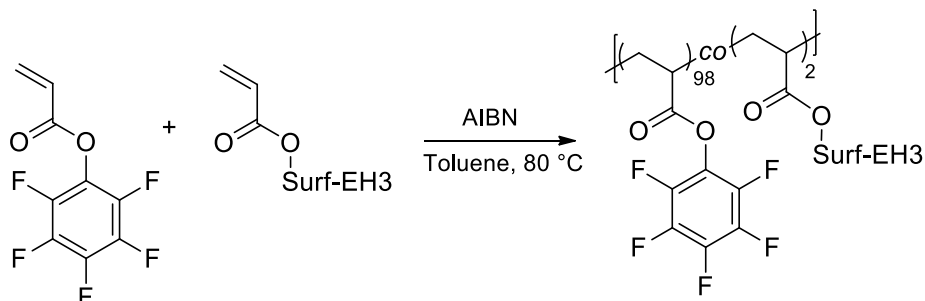
¹³C NMR (126 MHz, CDCl₃), δ ppm: δ 184.09 (s), 166.08 (s), 139.74 – 139.23 (m), 135.17 (m), 134.34 (s), 132.26 – 132.02 (m), 131.95 (s), 131.36 (s), 130.77 (s), 129.16 (s), 128.36 (s), 127.31 (m), 126.84 (s), 61.65 (s).

NMR spectra are found in Fig. SI 26, Fig. SI 27, Fig. SI 28 and Fig. SI 29 in appendix.

5.8 Synthesis of Polymers for Strategy 1

Synthesis of poly(pentafluorophenol-co-Surf-EH3A) (25)

The reaction was carried out according to a modified procedure from literature.[117]



PFPA 2.9 mL (17.6 mmol), Surf-EH3A 200 mg (0.36 mmol) and AIBN 30 mg (183 μ mol) were dissolved in 30 mL of Toluene. The solution was then purged with argon for 10 minutes and inserted into a preheated (80 °C) oil bath. The polymerization was terminated after 48 hours by purging with air and cooling to room temperature (20 °C). The polymer was first precipitated once in n-Heptane, redissolved in THF and reprecipitated in n-Heptane. After this a Liquid solid extraction in n-Heptane was performed and after centrifugation in n-Heptane the residual solvent was removed under vacuum.

Yield: 3.7 g (85 %).

Spectral data:

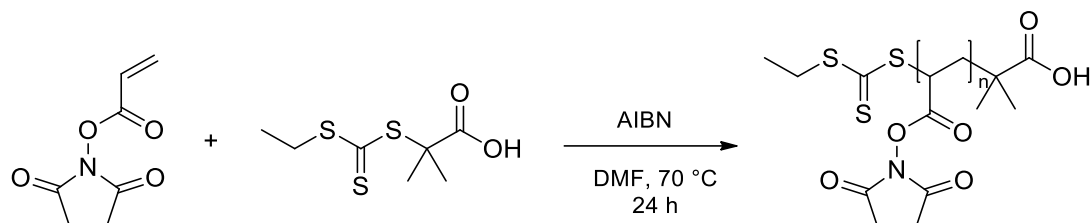
^1H NMR (500 MHz, CDCl_3), δ ppm: δ 3.69 – 3.24 (m, surfactant), 3.08 (br, s, 1H of the backbone), 2.56 – 2.04 (br, m, 2H of the backbone), 1.13 (m, surfactant), 0.92 – 0.78 (m, surfactant).

GPC: M_n : 20 kDa, Đ: 1.5.

NMR spectra is found in Fig. SI 30 in appendix.

Synthesis of polyN-hydroxysuccinimide acrylate

The following polymer was synthesized using reversible addition fragmentation chain transfer (RAFT) polymerization method following literature.[94,95,148]



NHSA (5 mmol, 0.85 g), 2-(ethylthiocarbonothioylthio)-2-methyl propanoic acid (EMP) (0.1 mmol, 22.4 mg), and AIBN (0.01 mmol, 1.6 mg) were dissolved in 10 mL of DMF under an inert atmosphere of argon (Ar). The flask was sealed and transferred to a pre-heated oil bath at 70°C. The reaction was allowed to run overnight. The flask was placed in a cold-water bath to terminate the reaction, and it was then opened to release any built-up pressure. The polymer was precipitated in cold methanol, filtered, and thoroughly washed with additional cold methanol to remove any unreacted monomers or byproducts. Finally, the polymer was dried under vacuum to obtain the final product.

Yield: 75%, 0.63 g, 3.73 mmol.

Spectral data:

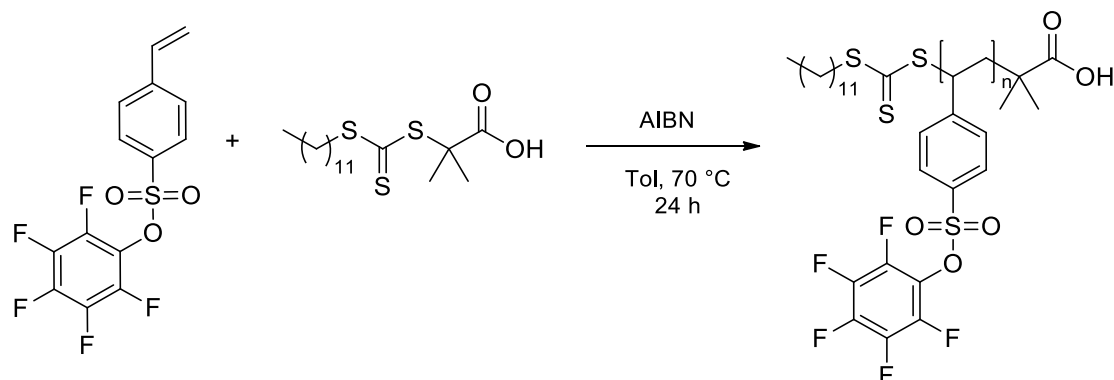
¹H-NMR (500 MHz, DMSO-*d*₆), δ ppm: δ 3.22 – 2.72 (m, protons of the NHS ring overlapping with the solvent DMF), 2.29 – 1.78 (m, backbone).

GPC: *M*_n: 50 kDa, Đ: 1.31.

NMR spectra is found in Fig. SI 31 in appendix.

Synthesis of polypentafluorophenol-4-vinylbenzene sulfonate

The following polymer was synthesized using reversible addition fragmentation chain transfer (RAFT) polymerization method following literature.[94,95,148]



PFP-4-VBS (427 mg, 1.22 mmol, 50 eq.), 2-[(Dodecylthio) carbonothioyl]thio-2-methylpropanoic acid (DMP) (9 mg, 0.025 mmol, 1 eq.), and AIBN (0.4 mg, 0.002 mmol, 0.1 eq. from stock solution 4 mg/20 μ L) were dissolved in 1.5 mL of toluene. The mixture was flushed with argon for 30 minutes and then heated in an oil bath to 70°C for 24 hours. The reaction was terminated by opening the vial, purging with air, and cooling the flask in a water bath. The polymer was precipitated in hexane (twice), centrifuged, and dried under high vacuum.

Yield: 51%, 0.63 mmol, 130 mg.

Spectral data:

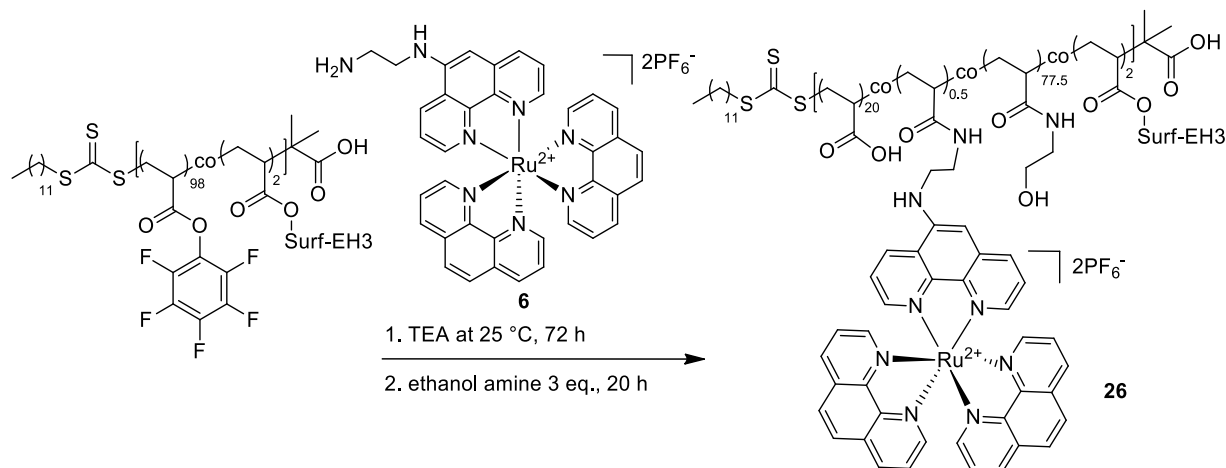
^1H NMR (500 MHz, CDCl_3), δ ppm: δ 8.21 – 6.53 (m, aromatic protons), 2.54 – 1.42 (m, backbone).

GPC: M_n : 12 kDa, Đ : 1.17.

NMR spectra is found in Fig. SI 32 in appendix.

Synthesis of poly(AA₂₀-co-Ru(NH₂)_{2.7}-co-HEAAm_{75.3}-co-SurfEH3A₂) (26**)**

The reaction was carried out according to a modified procedure from literature.[152,153]



The precursor polymer pPFPA₉₈-co-SurfEH3A₂ was synthesized via RAFT polymerization and provided by Karla Heckhäuser. This polymer retained the trithiocarbonate RAFT chain-end, which remained in the final modified polymer **26**.

To prepare the reaction mixture, 25 mg of **6** (0.04 mmol) was dissolved in 1.5 mL of *N,N*-dimethylformamide (DMF). In a separate flask, 102 mg of pPFPA-co-SurfEH3A (0.42 mmol) and 52 mg DMAP (1 mol eq.) were dissolved in 2 mL of DMF. Subsequently, the first solution was combined with the second one, and 70 mg of triethylamine (TEA) (0.67 mmol) was introduced into the resulting mixture. The reaction mixture was then stirred at 40 °C for a duration of 2 days. Following this initial period, 0.075 ml of ethanol amine (3 mol eq.) was added to the mixture, which was then stirred for an additional 24 hours at room temperature. The solvent was subsequently removed, and the remaining substance was dissolved in water. This solution was dialyzed using 2 kDa molar mass cutoff dialysis bags for a period of 2 days. After the dialysis, the solution was freeze-dried to obtain the final product.

Yield: 39 mg.

Spectral data:

¹H-NMR (500 MHz, DMSO-*d*₆), δ ppm: δ 8.84 – 7.6 (br, m, aromatic protons of [Ru]), 3.9 – 3.11 (br, m, -CH₂CH₂- of HEAAm overlapped with water peak at, ~3.3), 2.3 – 0.7 (br, m, backbone & surfactant).

UV-Vis abs.: $\lambda_{\text{max}} = 453 \text{ nm}$.

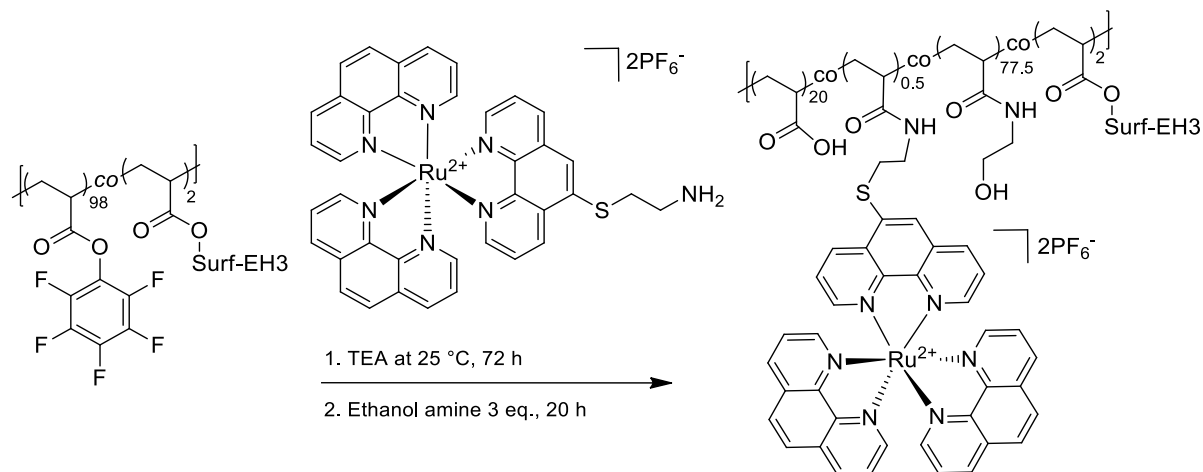
Phosphorescence emission: $\lambda_{\text{max}} = 590 \text{ nm}$.

GPC: $M_n = 6,5 \text{ kDa}$, $\bar{D} = 1.45$ The dispersity is somewhat higher than typical for well-controlled RAFT polymerizations (usually $\bar{D} < 1.3$), suggesting some loss of control during polymerization or potential side reactions.

NMR, UV-Vis absorption and emission spectra are found in Fig. SI 33 and Figure 38 in appendix and in results and discussion respectively.

Synthesis of poly(AA₂₀-co-Ru(S)_{0.5}-co-HEAAm_{77.5}-co-SurfEH3A₂) (27)

The reaction was carried out according to a modified procedure from literature.[152,153]



To prepare the reaction mixture, 30 mg of **8** (0.029 mmol) was dissolved in 1.5 mL of *N,N*-dimethylformamide (DMF). In a separate flask, 134 mg of pPFPA-co-SurfEH3A (0.56 mmol) was also dissolved in 2 mL of DMF. Subsequently, the first solution was combined with the second one, and 70 mg of triethylamine (TEA) (0.67 mmol) was introduced into the resulting mixture. The reaction mixture was then stirred at room temperature for a duration of 3 days. Following this initial period, 102 mg of ethanolamine (1.68 mmol, 3 eq.) was added to the mixture, which was then stirred for an additional 24 hours. The solvent was subsequently removed, and the remaining substance was dissolved in water. This solution was dialyzed against water using 2 kDa molar mass cutoff dialysis bags for a period of 2 days. After the dialysis, the solution was freeze-dried to obtain the final product.

Yield: 72 mg.

Spectral data:

¹H NMR (500 MHz, DMSO-*d*₆), δ ppm: δ 8.84 – 7.46 (br, m, aromatic protons of [Ru]), 3.67 – 2.64 (br, m, -CH₂CH₂- of HEAAm), 2.3 – 0.7 (br, m, backbone & surfactant).

UV-Vis abs.: λ_{max} = 453 nm.

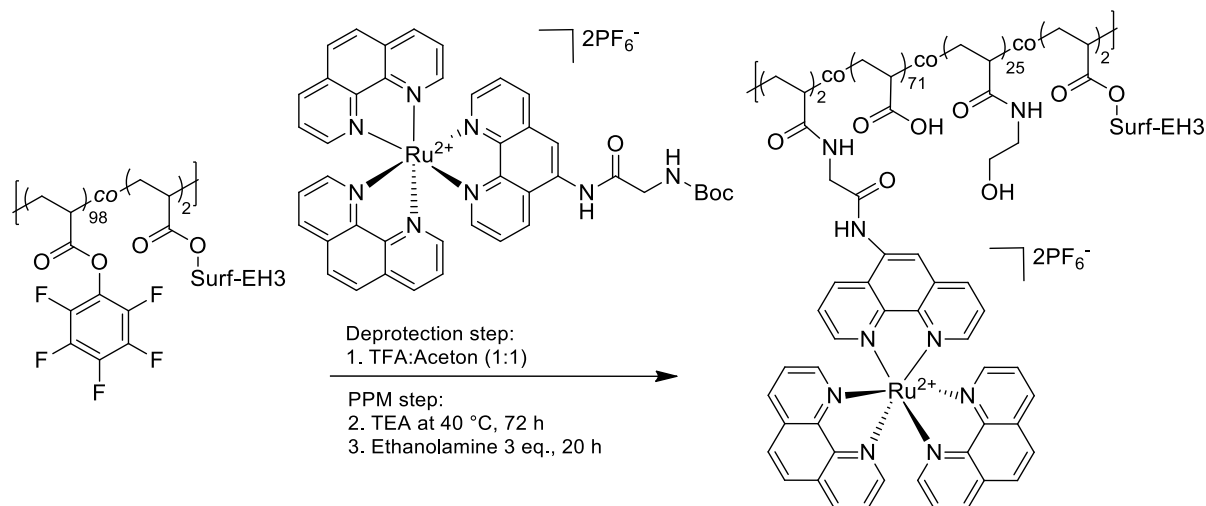
Phosphorescence emission: λ_{max} = 590 nm.

GPC: *M*_n: 10 kDa, Đ: 2.

NMR, UV-Vis absorption and emission spectra are found in Fig. SI 34 and Figure 38 in appendix and in results and discussions respectively.

Synthesis of poly(*Ru*(Boc)₂-co-AA₇₁-co-HEAAm₂₅-co-Surf-EH3A₂) (**28**)

The reaction was carried out according to a modified procedure from literature.[152,153]



In a 25 ml round-bottom flask, 54 mg (0.04 mmol) of **7** was dissolved in 2 ml of acetone. To this solution, 2 ml of trifluoroacetic acid (TFA) was added, and the mixture was stirred at room temperature overnight under a nitrogen atmosphere. After this period, the solvent was removed, and the product was used directly without further purification for the next step.

Next, 45 mg (0.045 mmol) of the product obtained from the previous step was dissolved in 2 ml of *N,N*-dimethylformamide (DMF). In a separate flask, 200 mg of pPFPA-co-SurfEH3 (0.85 mmol) was also dissolved in 2 ml of DMF. The solution containing the product was then combined with the solution of pPFPA-co-SurfEH3A, and 130 mg of triethylamine (TEA) (0.67 mmol) was introduced into the resulting mixture. The reaction mixture was stirred at 40 °C for a duration of 3 days. During this time, the solution became turbid, and some materials appeared to be insoluble, in contrast to the initial clear red solution.

Following this initial reaction period, 155 mg of ethanol amine (2.55 mmol, 3 equivalents) was added to the mixture at room temperature, and stirring was continued for an additional 24 hours. After the reaction was complete, the polymer was precipitated in acetone and centrifuged to yield an orange solid substance. This solid was then dissolved in water and dialyzed using 2 kDa molar mass cutoff dialysis bags

for a period of 3 days. Finally, the dialyzed solution was freeze-dried to obtain a fluffy orange solid.

Yield: 70 mg.

Spectral data:

^1H NMR (500 MHz, D_2O), δ ppm: 8.79 – 7.32 (br, m, aromatic protons of [Ru]), 3.79 – 3.24 (br, m, $-\text{CH}_2\text{CH}_2-$ of ethanol amine), 2.61 – 0.91 (br, m, backbone and surfactant).

^{19}F NMR (500 MHz, D_2O), δ ppm: No signals.

UV-Vis abs.: $\lambda_{\text{max}} = 453 \text{ nm}$

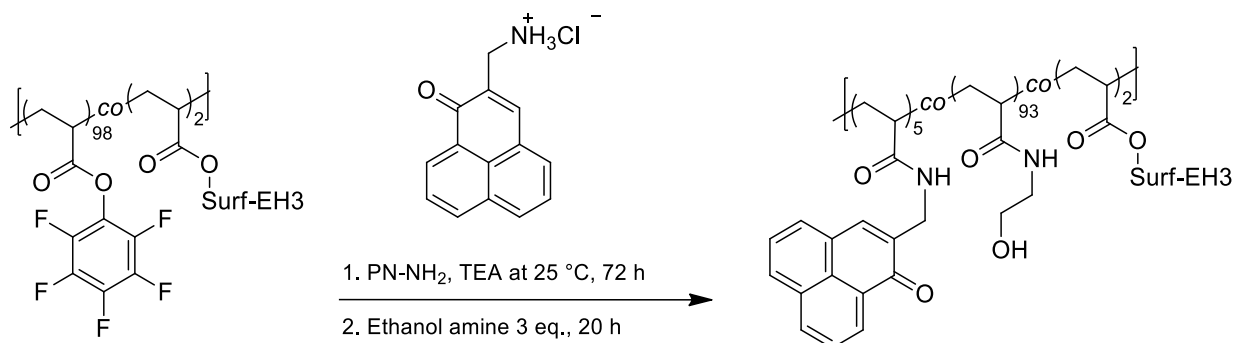
Phosphorescence emission: $\lambda_{\text{max}} = 590 \text{ nm}$

GPC: M_n : 12 kDa, Đ : 2.

NMR, UV-Vis absorption and emission spectra are found in Fig. SI 35, Fig. SI 36 and Figure 38 in appendix and in results and discussion respectively.

PPM reaction of PN-NH₂ with polyPFPA-co-SurfEH3A (29)

The reaction was carried out according to a modified procedure from literature.[152,153]



To prepare the reaction mixture for both the 5 mol% and 2 mol% grafting procedures, the following steps were undertaken. For the 5 mol% reaction, 12 mg of **9** (0.048 mmol, 5 mol%) was initially dissolved in 0.5 mL of methanol. This methanol was added after sequentially introducing 0.5 mL each of DMF, DCM, CHCl₃, and THF into the mixture. It is noteworthy that the material remained insoluble in these solvents except for methanol; however, the experiment proceeded as planned. In a separate flask, 238 mg of pPFPA-co-SurfEH3A (0.98 mmol) was dissolved in 2 mL of DMF. The first solution was then combined with the second one, followed by the addition of 118 mg of triethylamine (TEA) (1.175 mmol, 1.2 equivalents to the polymer) into the resulting mixture. This reaction mixture was stirred at room temperature for three days.

After this initial stirring period, 180 mg of ethanol amine (2.937 mmol, 3 equivalents) was introduced into the mixture, which was then stirred for an additional 24 hours. Subsequently, the solvent was removed, and the remaining substance was precipitated in water. The polymer was isolated through centrifugation and subsequently dissolved in dioxane before being freeze-dried to obtain the final product. It is important to note that the polymer exhibited insolubility in water, with phenalenone being grafted onto the polymer at an approximate ratio of 5 mol%.

Yield: 66 mg.

Spectral data:

^1H NMR (500 MHz, $\text{DMSO-}d_6$), δ ppm: δ 8.61 – 7.32 (m, aromatic protons of PN & amide), 4.80 – 4.51 (m, HO of HEAAm), 3.67 – 3.18 (m, $-\text{CH}_2\text{CH}_2-$ of HEAAm), 0.76 – 2.33 (m, backbone & surfactant).

GPC: M_n : 18 kDa, Đ : 1.8.

NMR spectra is found in Fig. SI 37 in appendix.

For the 2 mol% reaction, a similar approach was taken but with adjusted quantities. Here, 5 mg of **9** (0.02 mmol, 2 mol%) was dissolved in 0.5 mL of methanol, which was added after incorporating 2 mL of dioxane due to PN's poor solubility in dioxane. In a separate flask, again, 238 mg of pPFPA-co-SurfEH3A (0.98 mmol) was dissolved in 1 mL of dioxane. The first solution was combined with this second solution, followed by the addition of 118 mg of triethylamine (TEA) (1.175 mmol, 1.2 equivalents to the polymer). This mixture was also stirred at room temperature for three days.

Afterward, similar to the previous procedure, 180 mg of ethanol amine (2.937 mmol, 3 equivalents) was added and stirred for an additional 24 hours. The solvent was then partially removed before precipitating the remaining substance in ethyl acetate. The polymer underwent isolation by centrifugation and dissolution in dioxane before being freeze-dried to yield the final product.

Yield: 55 mg.

Spectral data:

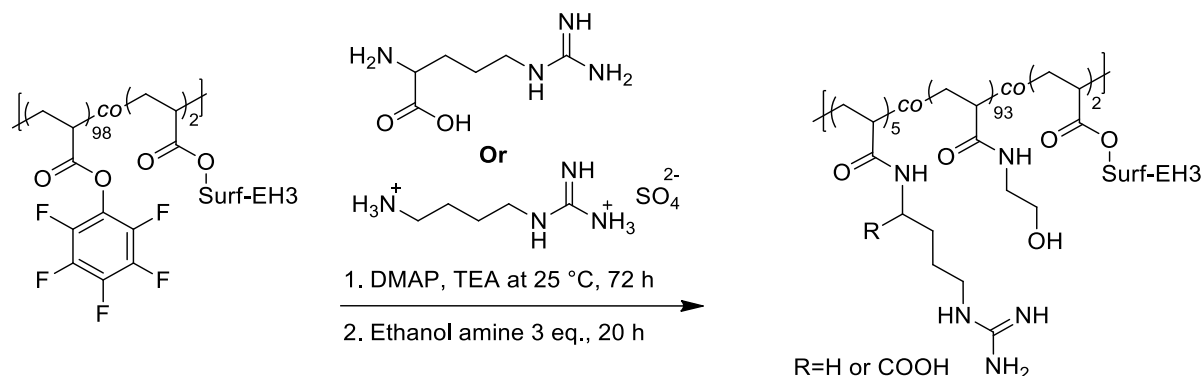
^1H -NMR (500 MHz, $\text{DMSO-}d_6$), δ ppm: 8.69 – 7.62 (m, aromatic protons of PN & amide), 4.85 – 4.50 (m, HO of HEAAm), 3.68 – 2.90 (m, $-\text{CH}_2\text{CH}_2-$ of HEAAm), 2.33 – 0.76 (m, backbone & surfactant).

GPC: M_n : 21 kDa, Đ : 1.6.

NMR spectra is found in Figure 27 in results and discussions.

PPM to introduce potential biofilm disruptor (no aPDT) (30,30a, 31 & 31a)

The reaction was carried out according to a modified procedure from literature.[152,153]



To prepare the reaction mixture, 33 mg (0.145 mmol) of agmatine sulphate (or 25 mg (0.145 mmol) of L-arginine) was dissolved in 1.5 mL of *N,N*-dimethylformamide (DMF). In a separate flask, 750 mg (3.18 mmol) of pPFPA-co-SurfEH3A and 9 mg DMAP (0.07 mmol) were dissolved in 7-8 mL of DMF. Subsequently, the first solution was combined with the second one, and 70 mg of triethylamine (TEA) (0.67 mmol) was introduced into the resulting mixture. The reaction mixture was then stirred at 25 °C for a duration of 2-3 days. Following this initial period, 0.5 ml of ethanol amine (8.28 mmol) was added to the mixture, which was then stirred for an additional 24 hours at room temperature. The polymer was isolated either by evaporation of the solvent or by precipitation in ethyl acetate. The isolated substance was dissolved in water. This solution was dialyzed using 2 kDa molar mass cutoff dialysis bags for a period of 2 days. After the dialysis, the solution was freeze-dried to obtain the final product.

- Poly (Agmatine₅-co-HEAAm₉₃-co-Surf-EH3A₂),

Yield: 404 mg.

Spectral data:

¹H NMR (500 MHz, D₂O), δ ppm: 7.29 – 7.05 (m, amide protons), 3.73 – 2.99 (br, m, -CH₂CH₂- of HEAAm), 2.79 (br S, agmatine), 2.53 – 0.63 (br, m, backbone and surfactant).

GPC: *M_n*: 8 kDa, Đ: 1.2.

Poly (Arginine₅-co-HEAAm₉₃-co-Surf-EH3A₂),

Yield: 487 mg.

Spectral data:

^1H NMR (500 MHz, D_2O), δ ppm: 7.29 – 7.05 (m, amide protons), 4.25 – 3.97 (m, arginine), 3.67 – 3.07 (br, m, $-\text{CH}_2\text{CH}_2-$ of HEAAm), 2.52 – 0.64 (br, m, backbone and surfactant).

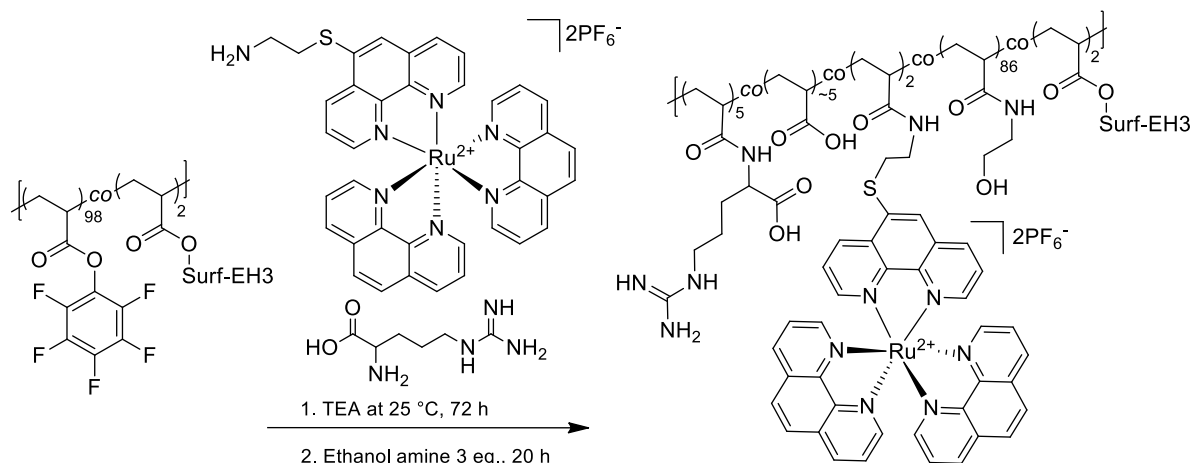
GPC: M_n : 8.8 kDa, Đ : 1.33.

The same polymers were synthesized using polymer **25a** (no surfactant) and the spectral data were similar.

NMR spectra are found in Fig. SI 38 and Fig. SI 39 in appendix.

PPM reaction to introduce Ru(S) and L-arginine (**32**)

The reaction was carried out according to a modified procedure from literature.[152,153]



To prepare the reaction mixture, 20 mg of **8** (2 mol%, 0.02 mmol) was dissolved in 0.5 mL of *N,N*-dimethylformamide (DMF). In a separate flask, 238 mg of pPFPA-co-SurfEH3A (0.979 mmol, PFPA part is 0.964) was also dissolved in 0.5 mL of DMF. Subsequently, the first solution was combined with the second one, and 70 mg of triethylamine (TEA) (0.67 mmol) was introduced into the resulting mixture. The reaction mixture was then stirred at room temperature for overnight.

Following this initial period, 8.4 mg (5 mol%, 0.048 mmol) of L-arginine was added and the mixture was stirred further for 24 hours at room temperature. On the third day, 102 mg of ethanol amine (1.68 mmol, 3 eq.) was dropped to the mixture, which was then stirred for an additional 24 hours. The solvent was subsequently removed, and the remaining substance was dissolved in water. This solution was dialyzed using 2 kDa molar mass cutoff dialysis bags for a period of 2 days. After the dialysis, the solution was freeze-dried to obtain the final product.

From the proton integrations on ^1H NMR, it is calculated that the polymer contains roughly 2 mol% Ruthenium complex and 5 mol% L-arginine.

Yield: 53 mg.

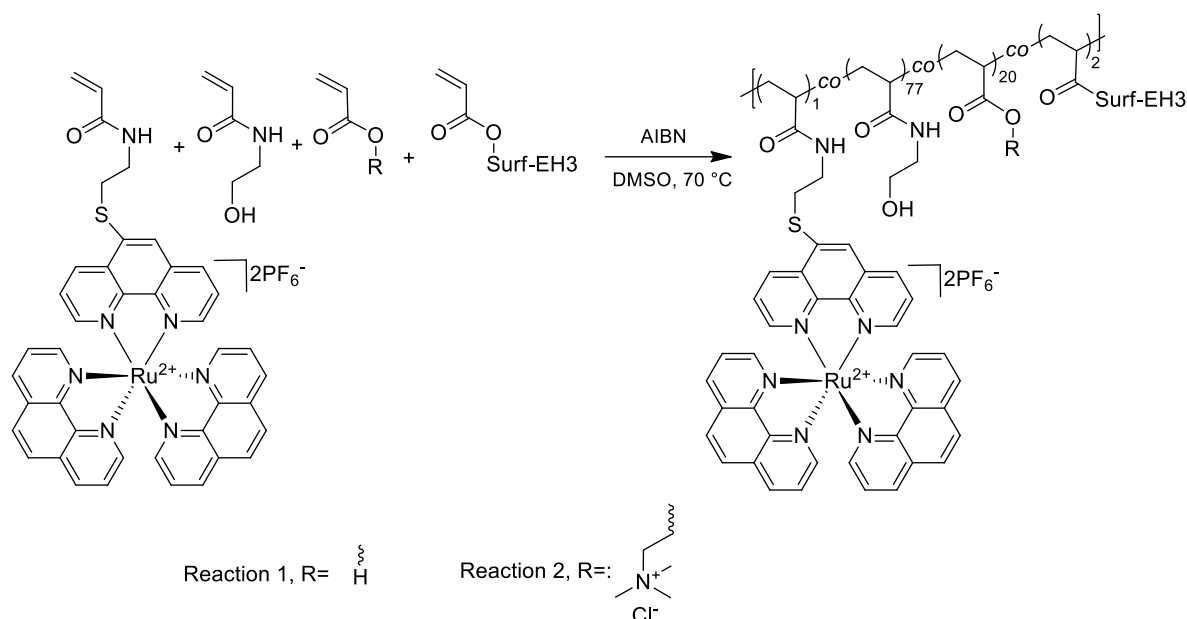
Spectral data:

^1H NMR (500 MHz, $\text{DMSO-}d_6$), δ ppm: δ 8.84 – 7.6 (br, m, aromatic protons of [Ru]), 4.75 – 4.5 (br, -CH- of L-Arginine) 3.7 – 3.24 (br, m, -CH₂CH₂- of HEAAm & H₂O), 2.4 – 0.7 (br, m, backbone & surfactant).

NMR spectra is found in Fig. SI 40 in appendix.

5.9 Synthesis of Polymers for Strategy 2

Synthesis of charged polymers (**33** and **33a**)



Feed in ratio for each component was as follows, Ru(S) (2 mol%), Surf-EH3A (2 mol%), azobisisobutyronitrile (AIBN, 1 mol%), HEAAm (76 mol%), and either:

- (a) Acrylic acid (20 mol%) for introducing negative charge (Reaction 1), or
- (b) [2-(acryloyloxy)ethyl]trimethylammonium chloride solution (AOETAC) (20 mol%) for introducing positive charge (Reaction 2)

were combined as follows:

Reaction 1 (**33**) (negative charge):

HEAAm (76 mg, 0.66 mmol), acrylic acid (12.7 mg, 0.18 mmol), Surf-EH3A (9.3 mg, 0.017 mmol), Ru(S) (18.7 mg, 0.017 mmol), and AIBN (1.4 mg, 0.009 mmol) were diluted with DMSO (0.3 mL) to give a total volume with the DMSO from the stock solutions of around 1 mL.

Reaction 2 (**33a**) (positive charge):

HEAAm (114 mg, 0.99 mmol), AOETAC (50 mg, 0.26 mmol), Surf-EH3A (14 mg, 0.026 mmol), Ru(S) (28 mg, 0.026 mmol), and AIBN (2 mg, 0.013 mmol) were diluted with DMSO (1.4 mL).

For both reactions: To remove any oxygen, three freeze-pump-thaw cycles were carried out. The solution was heated to 70 °C for one day. Afterwards, the polymer was precipitated in ice-cold EtOAc (220 mL) and centrifuged. The polymer was redissolved in water (2-3 mL), dialyzed (1 kDa) for 2 days, and freeze-dried to yield an orange polymer.

Yield:

Reaction 1 (**33**) (negative charge): 99%, 117 mg.

Reaction 2 (**33a**) (positive charge): 40%, 82 mg.

Spectral data:

Reaction 1 (**33**) (negative charge):

¹H NMR (500 MHz, DMSO-*d*₆), δ ppm: 8.84 – 8.25 (m, aromatic protons), 7.69 (m, amide protons of HEAAm), 4.24 – 3.99 (m, hydroxyl protons of HEAAm), 3.65 – 2.84 (m, -CH₂CH₂- of HEAAm), 2.27 – 0.76 (m, backbone & surfactant).

GPC: *M*_n: 22 kDa, Đ: 1.6.

UV-Vis abs.: λ_{max} = 453 nm.

Phosphorescence emission: λ_{max} = 590 nm.

Reaction 2 (**33a**) (positive charge):

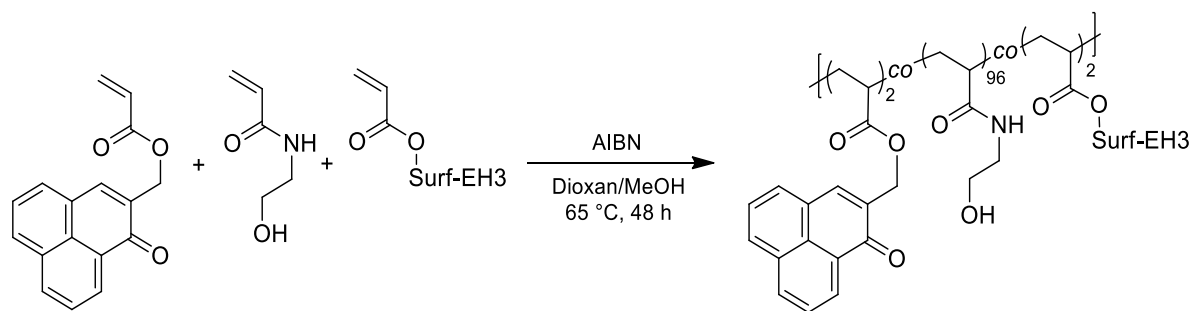
¹H NMR (500 MHz, DMSO-*d*₆), δ ppm: 8.92 – 7.64 (m, aromatic protons), 4.97 – 4.32 (m, hydroxyl protons of HEAAm and protons of AOETAC), 3.95 – 3.61 (m, AOETAC and surfactant), 3.57 – 3.21 (m, -CH₂CH₂- of HEAAm), 2.29 – 0.69 (m, backbone and surfactant).

GPC: Poorly soluble in DMAc.

UV-Vis abs.: λ_{max} = 453 nm.

NMR, UV-Vis absorption and emission spectra are found in Fig. SI 41, Fig. SI 42 and Figure 38 in appendix and in results and discussions respectively.

Synthesis of poly(*PN*₂-co-*HEA*Am₉₆-co-*Surf-EH3A*₂) (**35**)



Phenalenone acrylate (5.8 mg, 0.023 mmol, 2 mol%), HEAAm (122 mg, 1.06 mmol, 96 mol%), Surf-EH3A (12 mg, 0.023 mmol, 2 mol%) and AIBN (1.8 mg, 0.011 mmol, 1 mol%) were dissolved in a mixture of methanol/dioxane (0.8/0.5 mL) to give a total volume of 1.3 mL. To remove any oxygen, three freeze-pump-thaw cycles were carried out. The solution was heated to 65 °C for 48 h. Afterwards, the polymer was precipitated in ice-cold diethyl ether and centrifuged. The polymer was dried, redissolved in water, and dialyzed for one day to remove remaining monomers. The polymer was then obtained through lyophilization.

Yield: 57%, 80 mg.

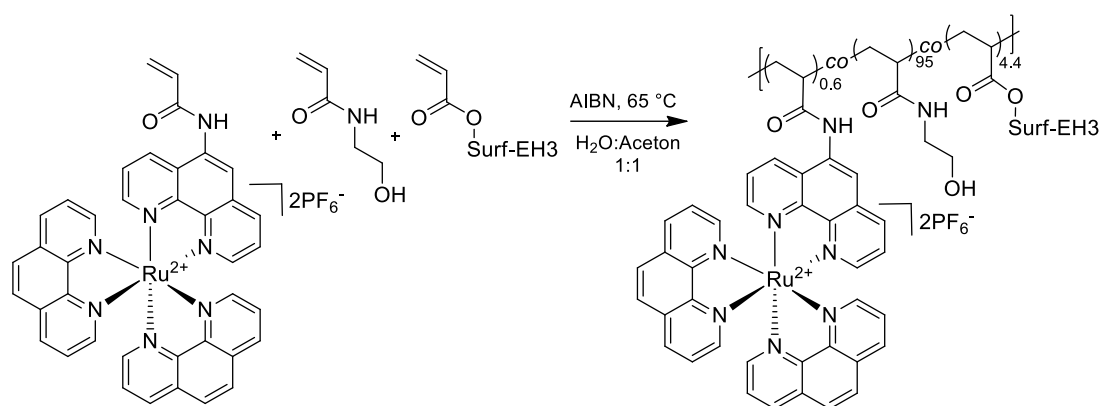
Spectral data:

¹H NMR (500 MHz, DMSO-*d*₆), δ ppm: 8.69 – 7.31 (m, amide and aromatic protons), 4.90 (m, hydroxyl protons of HEAAm), 3.62 – 2.81 (m, -CH₂CH₂- of HEAAm), 2.21 – 0.67 (m, backbone and surfactant).

UV-Vis abs.: λ_{max} = 363, 395 nm.

NMR and UV-Vis absorption spectra are found in Fig. SI 43 and Figure 32 in appendix and in results and discussions respectively.

Synthesis of poly($Ru(AA)_{0.6}$ -co-HEAAm₉₅-co-Surf-EH3A_{4.4}) (**36**)



11 (33 mg, 0.038 mmol), HEAAm (137 mg, 1.19 mmol), SurfEH3A (18 mg, 0.033 mmol) were dissolved in water/acetone (1/1) (4 mL). The polymerization mixture was degassed by argon purge, before AIBN (2 mg, 12 μ mol) was added under argon stream. The polymerization was started in a preheated oil bath at 65 °C under reflux. Since polyHEAAm is insoluble in acetone, it started precipitating during the reaction. Nevertheless, the polymerization was carried on and quenched after 24 hours by air under ice cooling. The solvents were evaporated, and diethyl ether was added to extract the unreacted monomers. After evaporation, the polymer was dissolved in water and dialyzed in 2 kDa bags for three days. The solution was turbid and not completely soluble. The sample was freeze-dried to obtain a solid yellow polymer. The availability of the complex unit was determined by ^1H NMR.

Yield: 51%, 78 mg.

Spectral data:

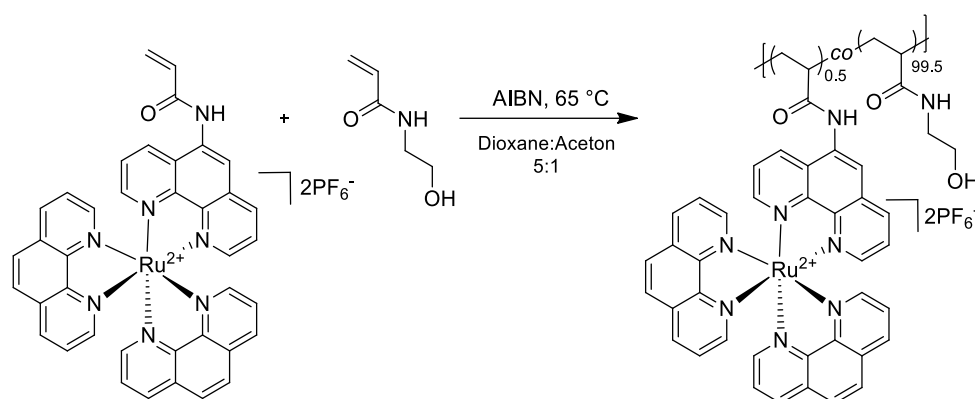
^1H NMR (500 MHz, DMSO d_6), δ ppm: δ 8.83 – 7.3 (br, m, aromatic protons of [Ru] and -NH- of HEAAm), 3.51 – 2.8 (br, m, -CH₂CH₂- of HEAAm), 2.2 – 0.6 (br, m, backbone & surfactant).

UV-Vis abs.: λ_{max} = 453 nm.

Phosphorescence emission: λ_{max} = 590 nm.

GPC: M_n : 10.6 kDa, $\bar{D}$: 1.9.

NMR, UV-Vis absorption and emission spectra are found in Fig. SI 44 and Figure 38 in appendix and results and discussions respectively.

Synthesis of poly($Ru(AA)_{0.5}$ -co-HEAAm_{99.5}) (**36a**)

11 (50 mg, 0.06 mmol) & HEAAm (181 mg, 1.57 mmol) were dissolved in Dioxane/Acetone (5/1) (6 mL). The polymerization mixture was degassed by argon purge, before AIBN (2 mg, 12 μ mol) was added under argon stream. The polymerization was started in a preheated oil bath at 65 °C under reflux. To avoid the precipitation of polyHEAAm during the reaction, 0.5 ml of DMSO was added. Nevertheless, the polymer partially precipitated but it was carried on and quenched after 24 hours by air under ice cooling. Most of the solvents were evaporated and 1 ml of DMSO was added to dissolve the precipitated polymer. The polymer was later precipitated in ethyl acetate, isolated, dissolved in water and dialyzed in 2 kDa bags for three days. The solution was clear. The sample was freeze-dried to obtain a solid yellow polymer. The availability of the complex unit was determined by ^1H NMR.

Yield: 40%, 77 mg.

Spectral data:

^1H NMR (500 MHz, D_2O), δ ppm: δ 8.78 – 7.43 (br, m, aromatic protons of [Ru]), 3.90 – 3.01 (br, 4H, m, $-\text{CH}_2\text{CH}_2-$ of HEAAm), 2.35 – 1.25 (br, 3H, m, backbone).

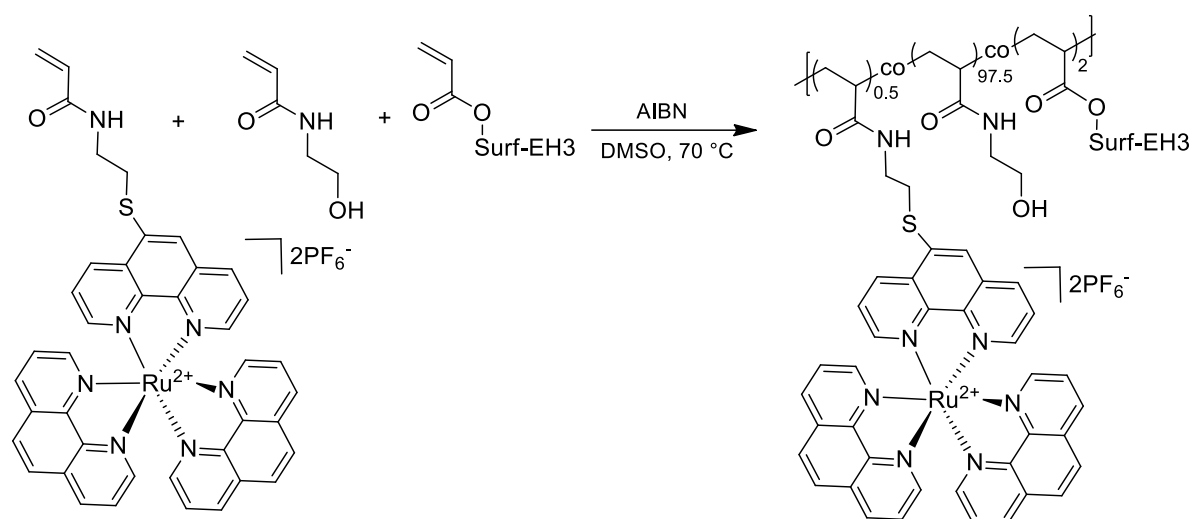
UV-Vis abs.: λ_{max} = 453 nm.

Phosphorescence emission: λ_{max} = 590 nm.

GPC: M_n : 8 kDa, Đ : 1.89.

NMR, UV-Vis absorption and emission spectra are found in Fig. SI 45 and Figure 38 in appendix and results and discussions respectively.

Synthesis of poly ($Ru(S)_{0.5}$ -co-HEAAm_{97.5}-co-SurfEH3A₂) (**37**)



HEAAm (144 mg, 1.25 mmol), Surf-EH3A (14 mg, 0.026 mmol, 2 mol%), **12** (28 mg, 0.026 mmol, 2 mol%) and AIBN (2.1 mg, 0.013 mmol, 1 mol%) were diluted with DMSO (1 mL) to give a total volume with the DMSO from the stock solutions of around 2.2 mL. To remove any oxygen, three freeze-pump-thaw cycles were carried out. The solution was heated to 70 °C overnight. Afterwards the polymer was precipitated in ice cold EtOAc (220 mL) and centrifuged. The polymer was redissolved in water and dialyzed (1 kDa) for two days and afterwards freeze dried to yield an orange polymer.

Yield: 65%, 122 mg.

Spectral data:

¹H NMR (500 MHz, DMSO-*d*₆), δ ppm: δ 8.89 – 8.31 (m, aromatic protons), 7.82 – 7.30 (m, amide protons of HEAAm), 4.90 (m, hydroxyl protons of HEAAm), 3.55 – 2.83 (m, ethyl protons of HEAAm), 2.09 – 1.24 (m, backbone), 1.11 – 0.72 (m, surfactant).

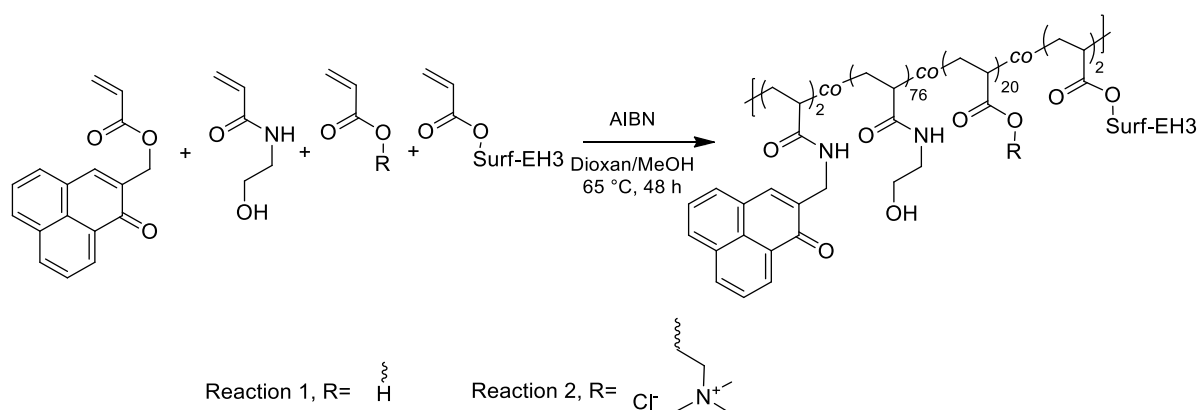
GPC: *M*_n: 19 kDa, Đ: 1.5.

UV-Vis abs.: λ_{max} = 453 nm.

Phosphorescence emission: λ_{max} = 590 nm.

NMR, UV-Vis absorption and emission spectra are found in Figure 33 and Figure 38 in results and discussions. The same synthetic procedure was followed for polymer **34**, yielding an orange polymer. The spectral characteristics were similar to those described above.

Synthesis of charged PN polymers



Phenalenone acrylate (5.8 mg, 0.023 mmol, 2 mol%), HEAAm (122 mg, 1.06 mmol, 76 mol%), Surf-EH3A (12 mg, 0.023 mmol, 2 mol%), AIBN (1.8 mg, 0.011 mmol, 1 mol%), and either:

(a) Acrylic acid (16 mg, 0.22 mmol, 20 mol%) for introducing negative charge (Reaction 1), or (b) [2-(acryloyloxy)ethyl]-trimethylammonium chloride (AOETAC) (43 mg, 0.22 mmol, 20 mol%) for introducing positive charge (Reaction 2).

were dissolved in a mixture of methanol/dioxane (0.8/0.5 mL) to give a total volume of 1.3 mL. To remove any oxygen, three freeze-pump-thaw cycles were carried out. The solution was heated to 65 °C for 48 h. Afterwards, the polymer was precipitated in ice-cold diethyl ether and centrifuged. The polymer was dried, redissolved in water, and dialyzed for one day to remove remaining monomers. The polymer was then obtained through lyophilization.

Yields:

Reaction 1 (negative charge): 57%, 74 mg.

Reaction 2 (positive charge): 51%, 80 mg.

Spectral data:

Reaction 1 (negative charge):

^1H NMR (500 MHz, $\text{DMSO}-d_6$), δ ppm: 8.68 – 7.26 (m, amide and aromatic protons), 5.27 – 4.72 (m, OH of HEAAm), 4.27 – 3.98 (m, Surfactant), 3.81 – 2.85 (m, $-\text{CH}_2\text{CH}_2-$ of HEAAm), 2.28 – 0.67 (m, backbone and surfactant).

GPC: M_n : 15 kDa, Đ : 1.5.

UV-Vis abs.: λ_{max} = 363, 395 nm.

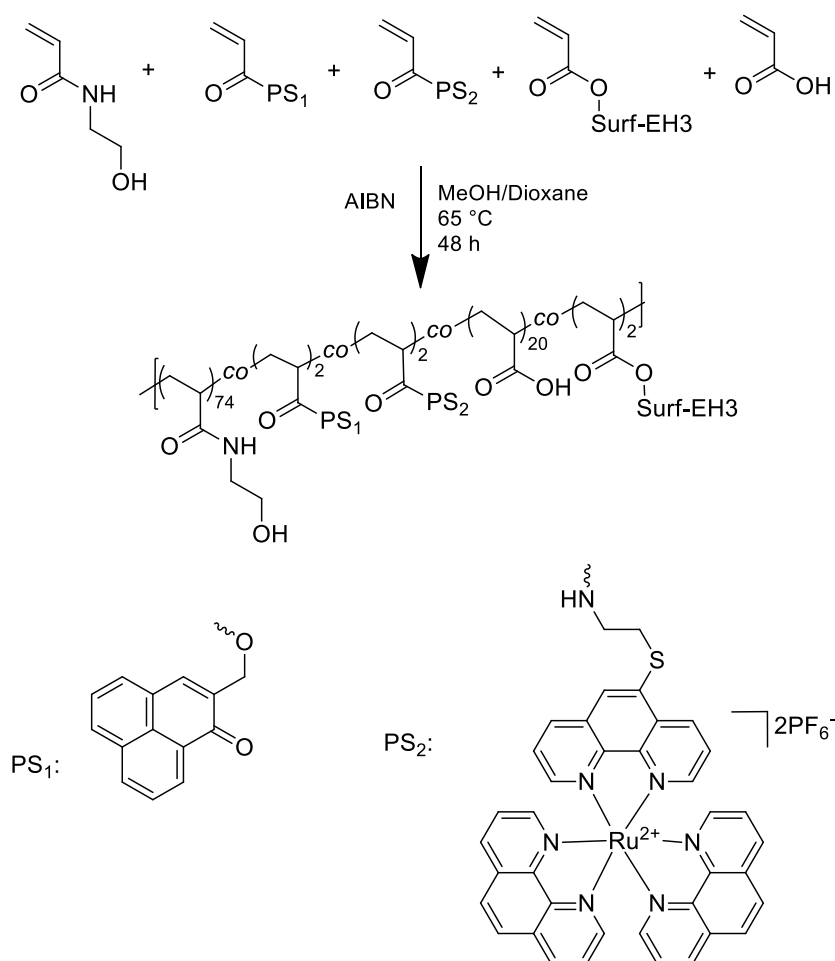
Reaction 2 (positive charge):

^1H NMR (500 MHz, DMSO- d_6), δ ppm: 8.92 – 7.45 (m, amide and aromatic protons), 5.27 – 4.34 (m, OH of HEAAm and AOETAC), 3.83 – 3.28 (m, Surfactant, AOETAC and $-\text{CH}_2\text{CH}_2-$ of HEAAm), 2.39 – 0.74 (m, backbone and surfactant).

UV-Vis abs.: λ_{max} = 363, 395 nm.

NMR spectra are found in Fig. SI 46 and Fig. SI 47 in appendix.

Synthesis of copolymer bearing two different photosensitizers.



PN (7 mg, 0.028 mmol, 2 mol%), Ru(S) (28 mg, 0.028 mmol, 2 mol%), acrylic acid (19 mg, 0.26 mmol, 20 mol%), Surf-EH3A (12 mg, 0.028 mmol, 2 mol%), HEAAm (111 mg, 0.97 mmol, 74 mol%), and AIBN (1.8 mg, 0.011 mmol, 1 mol%) were dissolved in a mixture of methanol/dioxane (1.2/0.3 mL) to give a total volume of 1.5 mL. To remove any oxygen, three freeze-pump-thaw cycles were carried out. The solution was heated to 65 °C for 48 h. Afterwards, the polymer was precipitated in ethyl acetate and centrifuged. The polymer was dried, redissolved in water, and dialyzed for one day to remove remaining monomers. The polymer was then obtained through lyophilization.

Yield: 30%, 54 mg.

Spectral data:

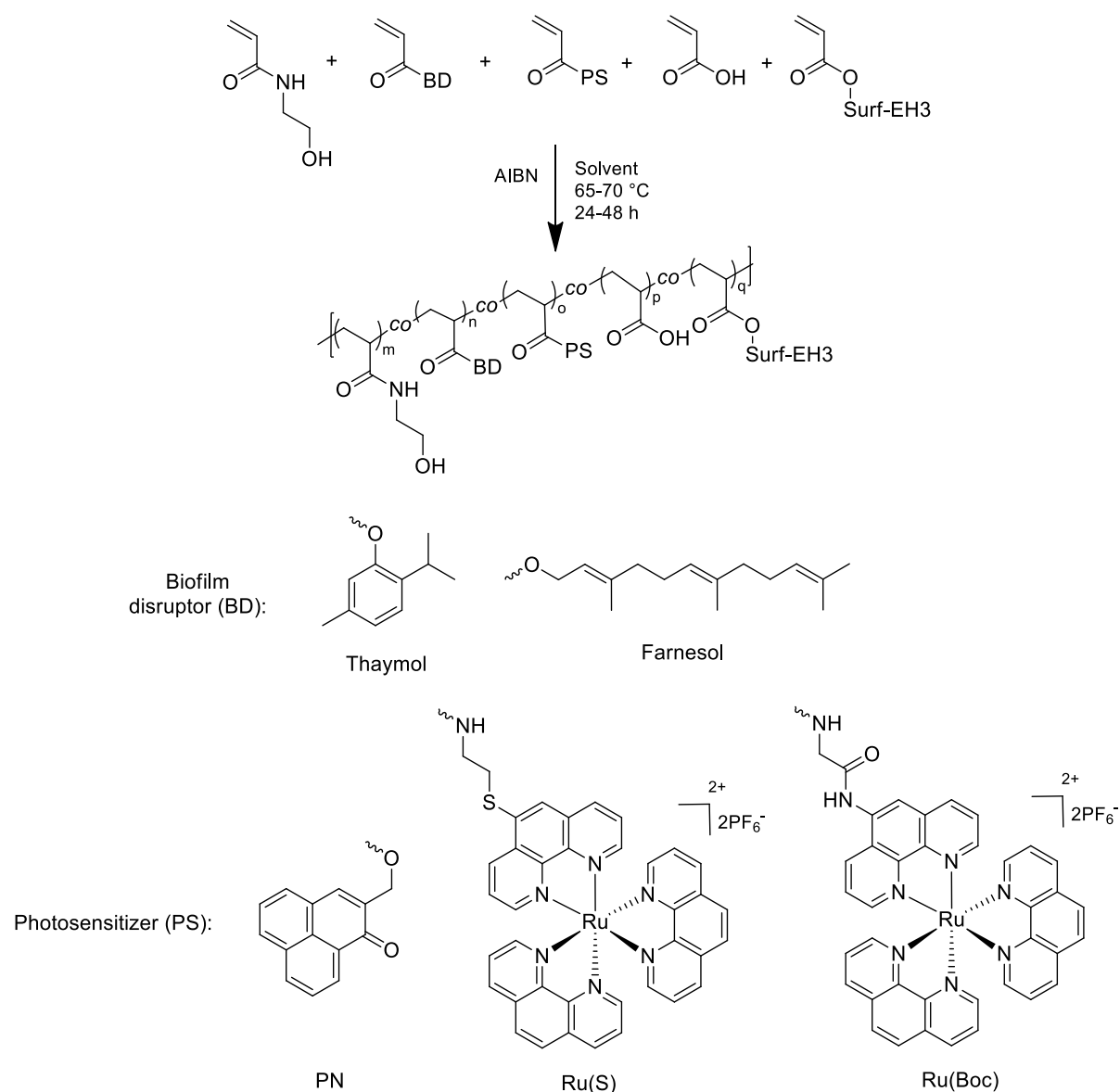
¹H NMR (500 MHz, DMSO-*d*₆), δ ppm: 9.15 – 7.35 (m, aromatic protons & amide protons of HEAAm), 3.65 – 2.78 (m, -CH₂CH₂- of HEAAm), 2.32 – 0.68 (m, backbone and surfactant).

GPC: M_n : 12.4 kDa, Đ: 1.24.

UV-Vis abs.: λ_{max} = 411 nm.

NMR and UV-Vis absorption spectra are found in Fig. SI 48 and Figure 32 in appendix and results and discussions respectively.

Synthesis of copolymers containing photosensitizer and biofilm disruptor



Polymerizations were performed by combining the components listed in Table SI 1 in the specified solvent. The mixture was deoxygenated using three freeze-pump-thaw cycles, then heated at the indicated temperature and time. The resulting polymers were precipitated in ice-cold ethyl acetate, except for S2 which was precipitated in diethylether. The polymers were dialyzed (1 kDa) for one day, and freeze-dried.

Table SI 1: Details of the conducted polymerizations.

S	PS (mol%/mg)	HEAAm (mol%/mg)	Acrylic acid (mol%/mg)	BD (mol%/mg)	Surf-EH3A (mol%/mg)	Solvent (mL)	t / T (d/°C)	Yield (mg/%)
---	-----------------	--------------------	------------------------------	-----------------	------------------------	-----------------	-----------------	-----------------

1	Ru(S) 2 / 28	91 / 136	-	Thymol 5 / 13	2 / 14	DMSO 2.1	1 / 70	93 / 49
2	Ru(S) 2 / 28	91 / 136	-	Farnesol 5 / 18	2 / 14	DMSO 2.1	1 / 70	98 / 50
3	Ru(S) 2 / 28	71 / 106	20 / 19	Thymol 5 / 13	2 / 14	DMSO 1.4	1 / 70	58 / 32
4	Ru(Boc) 2 / 28	91 / 136	-	Thymol 5 / 13	2 / 14	DMSO 2.0	1 / 70	108 / 57
5	PN- Acrylate 2 / 5.8	91 / 115	-	Thymol 5 / 11	2 / 12	MeOH/ Dioxane 0.9 / 0.5	2 / 65	84 / 58
6	PN- Acrylate 2 / 5.8	71 / 90	20 / 16	Farnesol 5 / 11	2 / 12	MeOH/ Dioxane 0.9 / 0.4	2 / 65	69 / 51

Spectral data:

S1:

¹H NMR (500 MHz, DMSO-*d*₆), δ ppm: 8.88 – 8.31 (m, aromatic protons of the complex), 8.11 – 7.44 (m, amide protons of HEAAm), 7.28 – 6.85 (m, protons of thymol), 5.15 – 4.50 (m, hydroxyl protons of HEAAm), 3.63 – 2.81 (m, -CH₂CH₂- of HEAAm), 2.37 – 0.73 (m, backbone & surfactant).

GPC: 20.1 *M*_n: kDa, Đ: 1.6.

UV-Vis abs.: λ_{max} = 453 nm.

NMR spectra is found in Fig. SI 49 in appendix.

S2:

¹H NMR (500 MHz, DMSO-*d*₆), δ ppm: 8.85 – 7.94 (m, aromatic protons of the complex), 7.84 – 7.42 (m, amide protons of HEAAm), 5.34 – 4.52 (m, protons of farnesol & hydroxyl protons of HEAAm), 3.64 – 2.78 (m, -CH₂CH₂- of HEAAm), 2.21 – 0.75 (m, backbone & surfactant).

GPC: *M*_n: 12.6 kDa, Đ: 1.69.

UV-Vis abs.: λ_{max} = 453 nm.

NMR spectra is found in Figure 35 in results and discussion.

S3:

^1H NMR (500 MHz, $\text{DMSO-}d_6$), δ ppm: 8.89 – 8.02 (m, aromatic protons of the complex), 7.94 – 7.43 (m, amide protons of HEAAm), 7.30 – 6.84 (m, protons of thymol), 3.60 – 2.90 (m, $-\text{CH}_2\text{CH}_2-$ of HEAAm), 2.25 – 0.71 (m, backbone & surfactant).

GPC: M_n : 18.6 kDa, Đ : 1.47.

UV-Vis abs.: λ_{max} = 453 nm.

NMR spectra is found in Fig. SI 50 in appendix.

S4:

^1H NMR (500 MHz, $\text{DMSO-}d_6$), δ ppm: 8.92 – 8.14 (m, aromatic protons of the complex), 8.03 – 7.43 (m, amide protons of HEAAm), 7.35 – 6.72 (m, protons of thymol), 5.17 – 4.57 (m, hydroxyl protons of HEAAm), 3.68 – 2.81 (m, $-\text{CH}_2\text{CH}_2-$ of HEAAm), 2.31 – 0.69 (m, backbone & surfactant).

GPC: M_n : 27.2 kDa, Đ : 1.62.

UV-Vis abs.: λ_{max} = 452 nm.

NMR spectra is found in Fig. SI 51 in appendix.

S5:

^1H NMR (500 MHz, $\text{DMSO-}d_6$), δ ppm: 8.74 – 8.19 (m, aromatic protons of phenalenone), 7.98 – 7.41 (m, amide protons of HEAAm), 7.24 – 6.81 (m, protons of thymol), 5.20 – 4.52 (m, hydroxyl protons of HEAAm), 3.62 – 2.77 (m, $-\text{CH}_2\text{CH}_2-$ of HEAAm), 2.35 – 0.63 (m, backbone & surfactant).

GPC: M_n : 19.5 kDa, Đ : 1.56.

UV-Vis abs.: λ_{max} = 363, 395 nm.

NMR spectra is found in Fig. SI 52 in appendix.

S6:

^1H NMR (500 MHz, $\text{DMSO-}d_6$), δ ppm: 8.62 – 8.19 (m, aromatic protons of phenalenone), 8.13 – 7.42 (m, amide protons of HEAAm), 7.29 – 6.82 (m, protons of

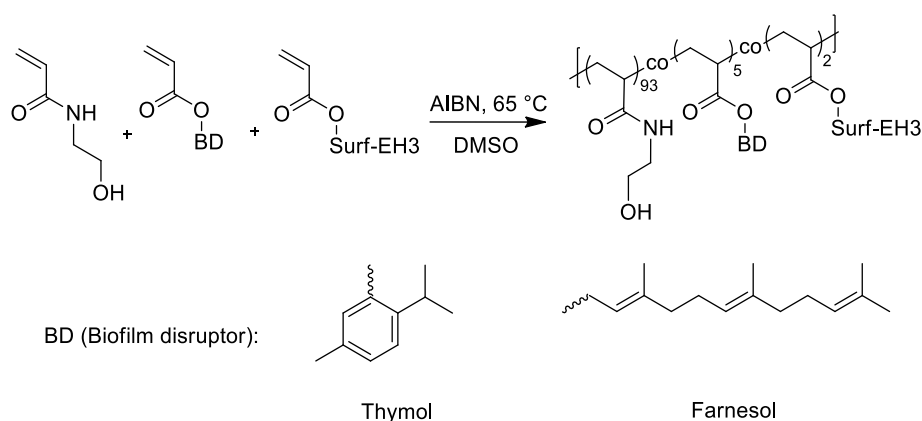
thymol), 5.30 – 4.54 (m, hydroxyl protons of HEAAm), 3.71 – 2.75 (m, -CH₂CH₂- of HEAAm), 2.32 – 0.69 (m, backbone & surfactant).

GPC: M_n : 17.5 kDa, Đ: 1.42.

UV-Vis abs.: λ_{max} = 363, 395 nm.

NMR spectra is found in Fig. SI 53 in appendix.

Polymers containing biofilm disruptors (no aPDT)



• Poly(Farnesol₅-co-HEAAm₉₃-co-Surf-EH3A₂) (**40**):

Farnesol acrylate (18 mg, 5 mol%), HEAAm (140 mg, 93 mol%), Surf-EH3A (14 mg, 2 mol%) and AIBN (1.8 mg, 0.011 mmol, 1 mol%) were dissolved in DMSO (2.2 mL). To remove any oxygen, three freeze-pump-thaw cycles were carried out. The solution was heated to 65-70 °C for 24 h. Afterwards, the polymer was precipitated in ice-cold diethyl ether and centrifuged. The polymer was dried, redissolved in water, and dialyzed for one day to remove remaining monomers. The polymer was then obtained through lyophilization.

Yield: 84%, 144 mg.

Spectral data:

¹H NMR (500 MHz, DMSO-*d*₆), δ ppm: 8.29 – 7.19 (m, amide and aromatic protons), 5.38 – 4.39 (m, Farnesol & hydroxyl protons of HEAAm), 3.66 – 2.82 (m, farnesol & -CH₂CH₂- of HEAAm), 2.14 – 0.60 (m, backbone and surfactant).

GPC: *M*_n: 33.2 kDa, Đ: 1.94.

NMR spectra is found in Fig. SI 54 in appendix.

• Poly(Thymol₅-co-HEAAm₉₃-co-Surf-EH3A₂) (**41**):

Thymol acrylate (46 mg, 0.22 mmol, 5 mol%), HEAAm (500 mg, 4.34 mmol, 93 mol%), Surf-EH3A (72 mg, 0.13 mmol, 2 mol%) and AIBN (8 mg, 0.07 mmol, 1.5 mol%) were dissolved in Toluene (5-7 mL). To remove any oxygen, three freeze-pump-thaw cycles were carried out. The solution was heated to 65 °C for 48 h. Afterwards, the polymer was precipitated in ice-cold ethyl acetate and centrifuged. The polymer was dried,

redissolved in water, and dialyzed for one day to remove remaining monomers. The polymer was then obtained through lyophilization.

Yield: 60%, 375 mg.

Spectral data:

^1H NMR (500 MHz, D_2O), δ ppm: 7.40 – 6.71 (m, aromatic protons of thymol), 3.85 – 3.02 (m, $-\text{CH}_2\text{CH}_2-$ of HEAAm), 2.47 – 0.77 (m, backbone and surfactant).

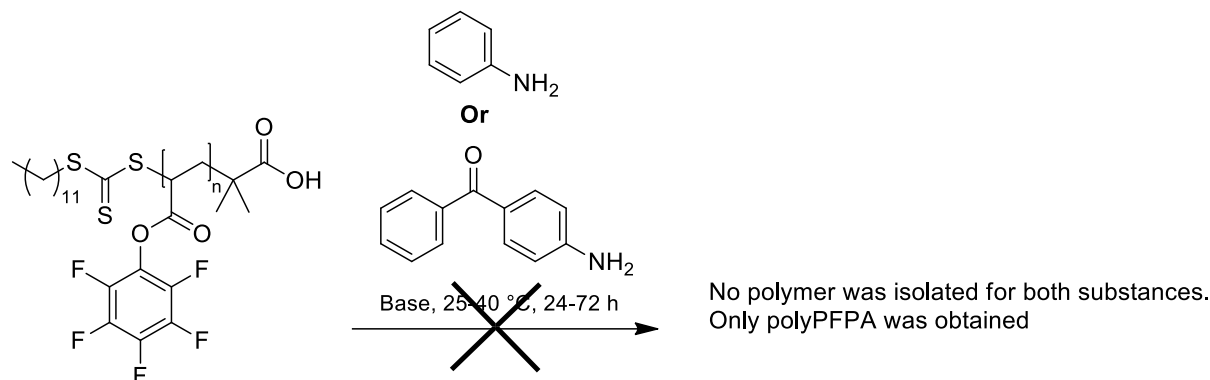
NMR spectra is found in Fig. SI 55 in appendix.

Both polymers were also synthesized without surfactant using the same procedure. Similar yields were obtained.

5.10 Failed Reactions

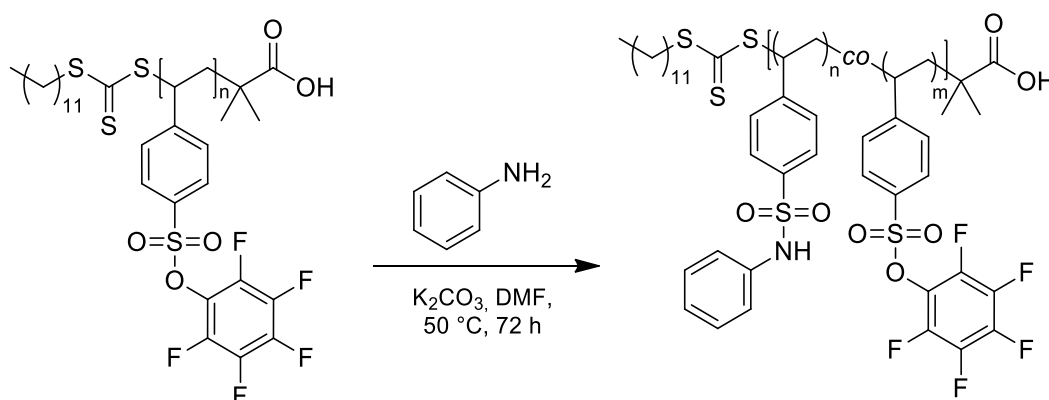
Introducing compounds bearing aromatic amines to the polymer

Investigation of PolyPFPA Reactivity with aromatic amines:



The potential modification of PolyPFPA using aniline was investigated. A standard synthetic protocol was employed, wherein 3-4 equivalents of the amine-containing compound were added to polyPFPA. It was observed that the reaction failed to produce the anticipated chemical transformation. Various alternative reaction conditions were systematically explored. Multiple base systems were evaluated, including triethylamine (TEA), potassium carbonate (K₂CO₃), and 4-dimethylaminopyridine (DMAP), each of which was selected to potentially catalyse the desired chemical modification. An alternative approach was also attempted by utilizing 4-aminobenzophenone as an alternative aromatic amine. Despite these comprehensive experimental approaches, no product could be isolated.

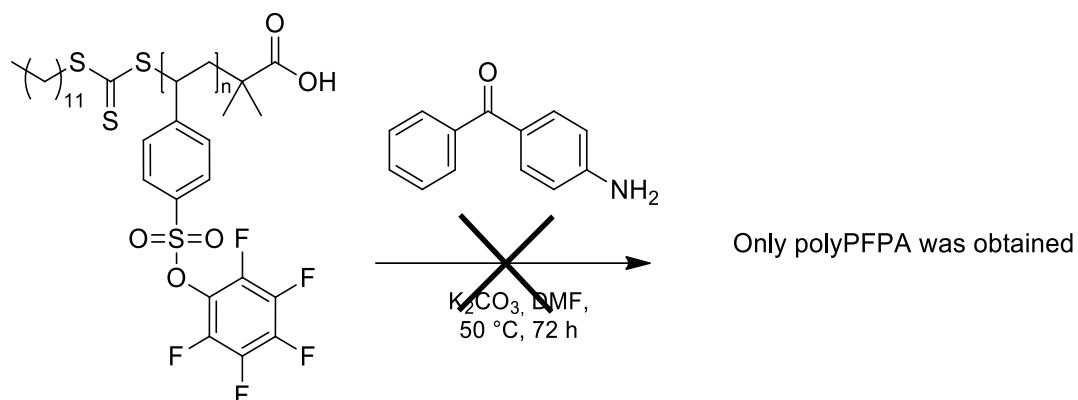
Investigation of PolyPFP4VBS Reactivity with aromatic amines:



In the subsequent experimental procedure, polyPFP4VBS, characterized by enhanced reactivity toward aromatic amines, was employed. The reaction was

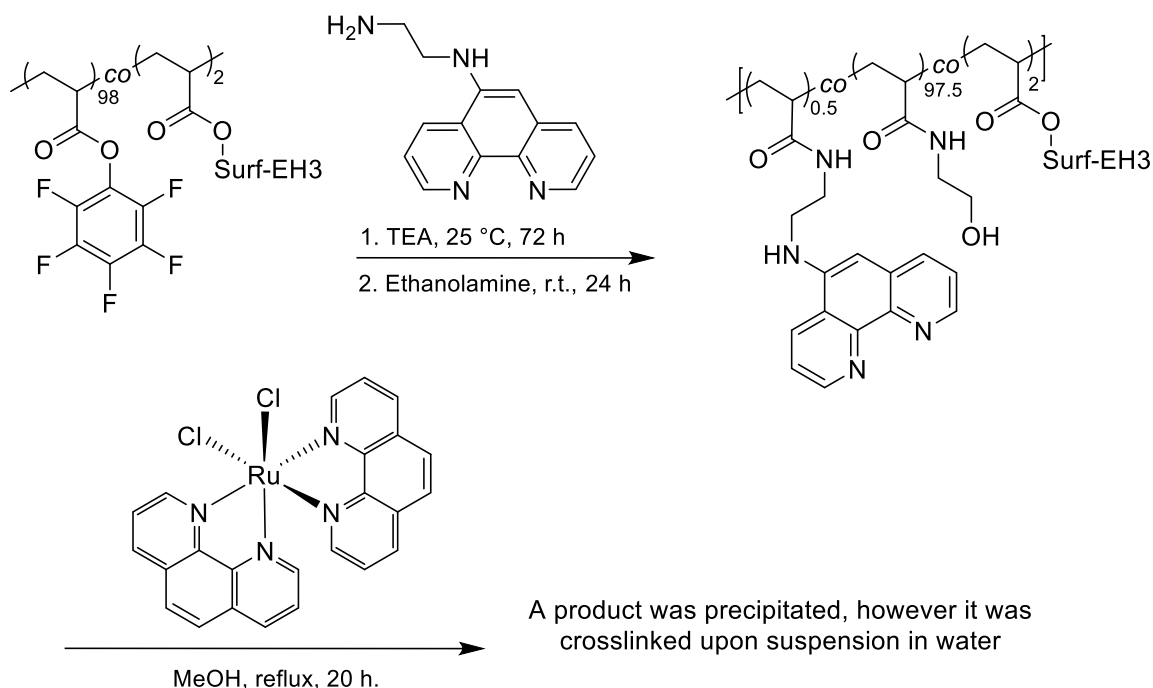
conducted using aniline to assess the potential for polymer modification. Partial modification of the active ester units was revealed, though the extent of transformation was considerably less than anticipated. An excess of aniline was deliberately added; however, only a fraction of the polymer was chemically modified.

Investigation with 4-Aminobenzophenone:



The reactivity of polyPFP4VBS with 4-aminobenzophenone was explored. Consistent with previous experimental attempts, the desired polymer modification was not achieved.

Unsuccessful building of the complex on the polymer using PPM



General procedure: PFPA-co-SurfEH3A was dissolved in THF (2 mL). N-(1,10-phenanthroline-5-yl)ethane-1,2-diamine (4) (7 mol%, 0.029 mmol) was dissolved in MeOH (~ 1.5 mL), and 4-(dimethylamino)pyridine (1 mol eq.) was added. The mixture was stirred at 35 °C for 24 h. It was then cooled to room temperature (~ 20 °C) before monoethanolamine (0.1 mL, 2 mol eq.) was added. After a further 24 hours, the solvent was removed under reduced pressure. The polymer was redissolved in a few mL of water, dialyzed in 1 kDa bags for 48 hours, and freeze-dried.

For the second step, the obtained polymer was dissolved in methanol. To this solution, 1 (5 mol eq. relative to the ligand content in the polymer) was added, and the reaction mixture was refluxed overnight. Afterwards, the solvent was removed under reduced pressure. The polymer was redissolved in a few mL of water, dialyzed for 48 hours, and freeze-dried. After this process, the polymer became insoluble in both organic and aqueous solvents, suggesting possible crosslinking. However, this is an assumption that requires confirmation. The reaction was repeated 4 times, consistently yielding the same outcome.

6 References

- [1] Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: A global multifaceted phenomenon. *Pathog Glob Health* 2015;109:309–18. <https://doi.org/10.1179/2047773215Y.0000000030>.
- [2] Walsh TR, Gales AC, Laxminarayan R, Dodd PC. Antimicrobial Resistance: Addressing a Global Threat to Humanity. *PLoS Med* 2023;20. <https://doi.org/10.1371/journal.pmed.1004264>.
- [3] Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, et al. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. *The Lancet* 2024;404:1199–226. [https://doi.org/10.1016/S0140-6736\(24\)01867-1](https://doi.org/10.1016/S0140-6736(24)01867-1).
- [4] Buckley GJ, Palmer GH. COMBATING ANTIMICROBIAL RESISTANCE AND PROTECTING THE MIRACLE OF MODERN MEDICINE. National Academies Press; 2022. <https://doi.org/10.17226/26350>.
- [5] Aslam B, Asghar R, Muzammil S, Shafique M, Siddique AB, Khurshid M, et al. AMR and Sustainable Development Goals: at a crossroads. *Global Health* 2024;20:1–21. <https://doi.org/10.1186/S12992-024-01046-8/FIGURES/13>.
- [6] Sadigov A, Mamedova I, Mammadov K. Ventilator-Associated Pneumonia and In-Hospital Mortality: Which Risk Factors may predict In-Hospital Mortality in Such Patients? vol. 3. 2019. <https://doi.org/10.29245/2689-999X/2019/4.1157>
- [7] Nelson RE, Hyun D, Jezek A, Samore MH. Mortality, Length of Stay, and Healthcare Costs Associated With Multidrug-Resistant Bacterial Infections Among Elderly Hospitalized Patients in the United States. *Clin Infect Dis* 2021;74:1070. <https://doi.org/10.1093/CID/CIAB696>.
- [8] Levy SB, Bonnie M. Antibacterial resistance worldwide: Causes, challenges and responses. *Nat Med* 2004;10:S122–9. <https://doi.org/10.1038/nm1145>.
- [9] Bender RG, Sirota SB, Swetschinski LR, Dominguez RMV, Novotney A, Wool EE, et al. Global, regional, and national incidence and mortality burden of non-COVID-19 lower respiratory infections and aetiologies, 1990–2021: a

- systematic analysis from the Global Burden of Disease Study 2021. *Lancet Infect Dis* 2024;24:974–1002. [https://doi.org/10.1016/S1473-3099\(24\)00176-2](https://doi.org/10.1016/S1473-3099(24)00176-2).
- [10] Albin OR, Admon AJ. Accurately measuring preventable ventilator-associated pneumonia deaths using observational data: It's about time. *Ann Am Thorac Soc* 2021;18:777–9. <https://doi.org/10.1513/AnnalsATS.202102-126ED>.
- [11] Pailhoriès H, Herrmann JL, Velo-Suarez L, Lamoureux C, Beauruelle C, Burgel PR, et al. Antibiotic resistance in chronic respiratory diseases: from susceptibility testing to the resistome. *European Respiratory Review* 2022;31. <https://doi.org/10.1183/16000617.0259-2021>.
- [12] Van Scott MR, Chandler J, Olmstead S, Brown JM, Mannie M. Airway anatomy, physiology, and inflammation. *The Toxicant Induction of Irritant Asthma, Rhinitis, and Related Conditions*, Springer US; 2013, p. 19–61. https://doi.org/10.1007/978-1-4614-9044-9_2.
- [13] Tosta E. The seven constitutive respiratory defense barriers against SARS-CoV-2 infection. *Rev Soc Bras Med Trop* 2021;54. <https://doi.org/10.1590/0037-8682-0461-2021>.
- [14] Gizurarson S. The Effect of Cilia and the Mucociliary Clearance on Successful Drug Delivery. vol. 38. 2015. <https://doi.org/10.1248/bpb.b14-00398>
- [15] Liu HY, Prentice EL, Webber MA. Mechanisms of antimicrobial resistance in biofilms. *Antimicrobials and Resistance* 2024;2:27. <https://doi.org/10.1038/s44259-024-00046-3>.
- [16] Boisvert AA, Cheng MP, Sheppard DC, Nguyen D. Microbial biofilms in pulmonary and critical care diseases. *Ann Am Thorac Soc* 2016;13:1615–23. <https://doi.org/10.1513/AnnalsATS.201603-194FR>.
- [17] Murgia X, De Souza Carvalho C, Lehr CM. Overcoming the pulmonary barrier: New insights to improve the efficiency of inhaled therapeutics. *Eur J Nanomed* 2014;6:157–69. <https://doi.org/10.1515/ejnm-2014-0019>.
- [18] He S, Gui J, Xiong K, Chen M, Gao H, Fu Y. A roadmap to pulmonary delivery strategies for the treatment of infectious lung diseases. *J Nanobiotechnology* 2022;20. <https://doi.org/10.1186/s12951-022-01307-x>.

- [19] Morrison CB, Markovetz MR, Ehre C. Mucus, mucins, and cystic fibrosis. *Pediatr Pulmonol* 2019;54:S84–96. <https://doi.org/10.1002/ppul.24530>.
- [20] Reihill JA, Douglas LEJ, Martin SL. Modulation of ion transport to restore airway hydration in cystic fibrosis. *Genes (Basel)* 2021;12. <https://doi.org/10.3390/genes12030453>.
- [21] Widdicombe JH. Regulation of the depth and composition of airway surface liquid. vol. 201. 2002. <https://doi.org/10.1046/j.1469-7580.2002.00098.x>
- [22] Martin LW, Gray AR, Brockway B, Lamont IL. *Pseudomonas aeruginosa* is oxygen-deprived during infection in cystic fibrosis lungs, reducing the effectiveness of antibiotics. *FEMS Microbiol Lett* 2023;370. <https://doi.org/10.1093/femsle/fnad076>.
- [23] Azoulay E, Russell L, Van de Louw A, Metaxa V, Bauer P, Povia P, et al. Diagnosis of severe respiratory infections in immunocompromised patients. *Intensive Care Med* 2020;46:298–314. <https://doi.org/10.1007/s00134-019-05906-5>.
- [24] Sanz-García F, Gil-Gil T, Laborda P, Ochoa-Sánchez LE, Martínez JL, Hernando-Amado S. Coming from the wild: Multidrug resistant opportunistic pathogens presenting a primary, not human-linked, environmental habitat. *Int J Mol Sci* 2021;22. <https://doi.org/10.3390/ijms22158080>.
- [25] Huemer M, Mairpady Shambat S, Brugger SD, Zinkernagel AS. Antibiotic resistance and persistence—Implications for human health and treatment perspectives. *EMBO Rep* 2020;21. <https://doi.org/10.15252/embr.202051034>.
- [26] Meers P, Neville M, Malinin V, Scotto AW, Sardaryan G, Kurumunda R, et al. Biofilm penetration, triggered release and in vivo activity of inhaled liposomal amikacin in chronic *Pseudomonas aeruginosa* lung infections. *Journal of Antimicrobial Chemotherapy* 2008;61:859–68. <https://doi.org/10.1093/jac/dkn059>.
- [27] Cazzola M, Matera MG, Noschese P. Parenteral antibiotic therapy in the treatment of lower respiratory tract infections. Strategies to minimize the

- development of antibiotic resistance. *Pulm Pharmacol Ther* 2000;13:249–56. <https://doi.org/10.1006/pupt.2000.0253>.
- [28] Smith DA, Beaumont K, Maurer TS, Di L. Clearance in Drug Design. *J Med Chem* 2019;62:2245–55. <https://doi.org/10.1021/acs.jmedchem.8b01263>.
- [29] Sou T, Kukavica-Ibrulj I, Soukarieh F, Halliday N, Levesque RC, Williams P, et al. Model-Based Drug Development in Pulmonary Delivery: Pharmacokinetic Analysis of Novel Drug Candidates for Treatment of *Pseudomonas aeruginosa* Lung Infection. *J Pharm Sci* 2019;108:630–40. <https://doi.org/10.1016/j.xphs.2018.09.017>.
- [30] Hol FJH, Hubert B, Dekker C, Keymer JE. Density-dependent adaptive resistance allows swimming bacteria to colonize an antibiotic gradient. *ISME Journal* 2016;10:30–8. <https://doi.org/10.1038/ismej.2015.107>.
- [31] Hermesen R, Deris JB, Hwa T. On the rapidity of antibiotic resistance evolution facilitated by a concentration gradient n.d. <https://doi.org/10.1073/pnas.1117716109>.
- [32] Wang T, Rong F, Tang Y, Li M, Feng T, Zhou Q, et al. Targeted polymer-based antibiotic delivery system: A promising option for treating bacterial infections via macromolecular approaches. *Prog Polym Sci* 2021;116. <https://doi.org/10.1016/j.progpolymsci.2021.101389>.
- [33] Moreno-Sastre M, Pastor M, Salomon CJ, Esquisabel A, Pedraz JL. Pulmonary drug delivery: A review on nanocarriers for antibacterial chemotherapy. *Journal of Antimicrobial Chemotherapy* 2015;70:2945–55. <https://doi.org/10.1093/jac/dkv192>.
- [34] Langendonk RF, Neill DR, Fothergill JL. The Building Blocks of Antimicrobial Resistance in *Pseudomonas aeruginosa*: Implications for Current Resistance-Breaking Therapies. *Front Cell Infect Microbiol* 2021;11. <https://doi.org/10.3389/fcimb.2021.665759>.
- [35] Maurice NM, Bedi B, Sadikot RT. *Pseudomonas aeruginosa* biofilms: Host response and clinical implications in lung infections. *Am J Respir Cell Mol Biol* 2018;58:428–39. <https://doi.org/10.1165/rcmb.2017-0321TR>.

- [36] WHO publishes list of bacteria for which new antibiotics are urgently needed n.d. <https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed> (accessed January 26, 2025).
- [37] Davies JC. *Pseudomonas aeruginosa* in cystic fibrosis: Pathogenesis and persistence. *Paediatr Respir Rev* 2002;3:128–34. [https://doi.org/10.1016/S1526-0550\(02\)00003-3](https://doi.org/10.1016/S1526-0550(02)00003-3).
- [38] Fischer AJ, Singh SB, LaMarche MM, Maakestad LJ, Kienenberger ZE, Peña TA, et al. Sustained Coinfections with *Staphylococcus aureus* and *Pseudomonas aeruginosa* in Cystic Fibrosis. *Am J Respir Crit Care Med* 2021;203:328–38. <https://doi.org/10.1164/RCCM.202004-1322OC>.
- [39] Reyne N, McCarron A, Cmielewski P, Parsons D, Donnelley M. To bead or not to bead: A review of *Pseudomonas aeruginosa* lung infection models for cystic fibrosis. *Front Physiol* 2023;14:1104856. <https://doi.org/10.3389/FPHYS.2023.1104856>.
- [40] Ciofu O, Riis B, Pressler T, Poulsen HE, Høiby N. Occurrence of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis patients is associated with the oxidative stress caused by chronic lung inflammation. *Antimicrob Agents Chemother* 2005;49:2276–82. <https://doi.org/10.1128/AAC.49.6.2276-2282.2005>.
- [41] Malhotra S, Hayes D, Wozniak DJ. Cystic Fibrosis and *Pseudomonas aeruginosa*: the Host-Microbe Interface. *Clin Microbiol Rev* 2019;32:e00138-18. <https://doi.org/10.1128/CMR.00138-18>.
- [42] Murray TS, Egan M, Kazmierczak BI. *Pseudomonas aeruginosa* chronic colonization in cystic fibrosis patients. *Curr Opin Pediatr* 2007;19:83–8. <https://doi.org/10.1097/MOP.0B013E3280123A5D>.
- [43] Fothergill JL, Neill DR, Loman N, Winstanley C, Kadioglu A. *Pseudomonas aeruginosa* adaptation in the nasopharyngeal reservoir leads to migration and persistence in the lungs. *Nat Commun* 2014;5. <https://doi.org/10.1038/ncomms5780>.

- [44] Ciofu O, Tolker-Nielsen T. Tolerance and resistance of pseudomonas aeruginosabiofilms to antimicrobial agents-how P. aeruginosaCan escape antibiotics. *Front Microbiol* 2019;10. <https://doi.org/10.3389/fmicb.2019.00913>.
- [45] Li X, Gu N, Huang TY, Zhong F, Peng G. Pseudomonas aeruginosa: A typical biofilm forming pathogen and an emerging but underestimated pathogen in food processing. *Front Microbiol* 2023;13:1114199. <https://doi.org/10.3389/FMICB.2022.1114199>.
- [46] Reig S, Le Gouellec A, Bleves S. What Is New in the Anti–Pseudomonas aeruginosa Clinical Development Pipeline Since the 2017 WHO Alert? *Front Cell Infect Microbiol* 2022;12:909731. <https://doi.org/10.3389/FCIMB.2022.909731>.
- [47] Niculescu AG, Grumezescu AM. Polymer-Based Nanosystems—A Versatile Delivery Approach. *Materials* 2021;14:6812. <https://doi.org/10.3390/MA14226812>.
- [48] Kumar M, Hilles AR, Almurisi SH, Bhatia A, Mahmood S. Micro and nano-carriers-based pulmonary drug delivery system: Their current updates, challenges, and limitations – A review. *JCIS Open* 2023;12:100095. <https://doi.org/10.1016/J.JCISO.2023.100095>.
- [49] Principi N, Silvestri E, Esposito S. Advantages and limitations of bacteriophages for the treatment of bacterial infections. *Front Pharmacol* 2019;10:457104. <https://doi.org/10.3389/FPHAR.2019.00513>.
- [50] Cieplik F, Deng D, Crielaard W, Buchalla W, Hellwig E, Al-Ahmad A, et al. Antimicrobial photodynamic therapy – what we know and what we don't. *Crit Rev Microbiol* 2018;44:571–89. <https://doi.org/10.1080/1040841X.2018.1467876>.
- [51] Youf R, Müller M, Balasini A, Thétiot F, Müller M, Hascoët A, et al. Antimicrobial photodynamic therapy: Latest developments with a focus on combinatory strategies. *Pharmaceutics* 2021;13. <https://doi.org/10.3390/pharmaceutics13121995>.

- [52] Kick G, Messer G, Plewig G. Historische Entwicklung der Photodynamischen Therapie. *Hautarzt* 1996;47:644–9. <https://doi.org/10.1007/S001050050485>.
- [53] van Straten D, Mashayekhi V, de Bruijn HS, Oliveira S, Robinson DJ. Oncologic Photodynamic Therapy: Basic Principles, Current Clinical Status and Future Directions. *Cancers (Basel)* 2017;9:19. <https://doi.org/10.3390/CANCERS9020019>.
- [54] Cieplik F, Deng D, Crielaard W, Buchalla W, Hellwig E, Al-Ahmad A, et al. Antimicrobial photodynamic therapy—what we know and what we don't. *Crit Rev Microbiol* 2018;44:571–89. <https://doi.org/10.1080/1040841X.2018.1467876>.
- [55] Abrahamse H, Hamblin MR. New photosensitizers for photodynamic therapy. *Biochem J* 2016;473:347. <https://doi.org/10.1042/BJ20150942>.
- [56] Sun X, Sun J, Sun Y, Li C, Fang J, Zhang T, et al. Oxygen Self-Sufficient NanoplatforM for Enhanced and Selective Antibacterial Photodynamic Therapy against Anaerobe-Induced Periodontal Disease. *Adv Funct Mater* 2021;31:2101040. <https://doi.org/10.1002/ADFM.202101040>.
- [57] Chang H, Liao KS, Hsieh YS. Bronchoscopic light delivery method for peripheral lung cancer photodynamic therapy. *J Thorac Dis* 2020;12:3611. <https://doi.org/10.21037/JTD-19-3887>.
- [58] Zhao H, Wang X, Xiong J, Liang G, Wu X, Xi J, et al. Miniaturized all-in-one microneedle device for point of care light therapy. *Npj Flexible Electronics* 2024 8:1 2024;8:1–11. <https://doi.org/10.1038/s41528-024-00317-z>.
- [59] Li H, Zhou X, Huang Y, Liao B, Cheng L, Ren B. Reactive Oxygen Species in Pathogen Clearance: The Killing Mechanisms, the Adaption Response, and the Side Effects. *Front Microbiol* 2021;11:622534. <https://doi.org/10.3389/FMICB.2020.622534>.
- [60] Alfei S, Schito GC, Schito AM, Zuccari G. Reactive Oxygen Species (ROS)-Mediated Antibacterial Oxidative Therapies: Available Methods to Generate ROS and a Novel Option Proposal. *Int J Mol Sci* 2024;25:7182. <https://doi.org/10.3390/IJMS25137182>.

- [61] Juan CA, de la Lastra JMP, Plou FJ, Pérez-Lebeña E. The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. *International Journal of Molecular Sciences* 2021, Vol 22, Page 4642 2021;22:4642. <https://doi.org/10.3390/IJMS22094642>.
- [62] Mahmoudi H, Bahador A, Pourhajibagher M, Alikhani MY. Antimicrobial Photodynamic Therapy: An Effective Alternative Approach to Control Bacterial Infections. *J Lasers Med Sci* 2018;9:154. <https://doi.org/10.15171/JLMS.2018.29>.
- [63] Klausen M, Ucuncu M, Bradley M. Design of Photosensitizing Agents for Targeted Antimicrobial Photodynamic Therapy. *Molecules* 2020, Vol 25, Page 5239 2020;25:5239. <https://doi.org/10.3390/MOLECULES25225239>.
- [64] Yu Q, Wang C, Zhang X, Chen H, Wu MX, Lu M. Photochemical Strategies toward Precision Targeting against Multidrug-Resistant Bacterial Infections. *ACS Nano* 2024;18:14085–122. <https://doi.org/10.1021/acsnano.3c12714>.
- [65] Hu X, Zhang H, Wang Y, Shiu BC, Lin JH, Zhang S, et al. Synergistic antibacterial strategy based on photodynamic therapy: Progress and perspectives. *Chemical Engineering Journal* 2022;450:138129. <https://doi.org/10.1016/J.CEJ.2022.138129>.
- [66] Martins Antunes de Melo W de C, Celiešiūtė-Germanienė R, Šimonis P, Stirkė A. Antimicrobial photodynamic therapy (aPDT) for biofilm treatments. Possible synergy between aPDT and pulsed electric fields. *Virulence* 2021;12:2247. <https://doi.org/10.1080/21505594.2021.1960105>.
- [67] Wang Z, Xu FJ, Yu B. Smart Polymeric Delivery System for Antitumor and Antimicrobial Photodynamic Therapy. *Front Bioeng Biotechnol* 2021;9:783354. <https://doi.org/10.3389/FBIOE.2021.783354>.
- [68] Kaur G, Narang RK, Rath G, Goyal AK. Advances in Pulmonary Delivery of Nanoparticles. *Artificial Cells, Blood Substitutes, and Biotechnology* 2012;40:75–96. <https://doi.org/10.3109/10731199.2011.592494>.

- [69] Gonsalves A, Menon JU. Impact of Nebulization on the Physicochemical Properties of Polymer–Lipid Hybrid Nanoparticles for Pulmonary Drug Delivery. *Int J Mol Sci* 2024;25:5028. <https://doi.org/10.3390/IJMS25095028>.
- [70] Wu G, Xu Z, Yu Y, Zhang M, Wang S, Duan S, et al. Biomaterials-based phototherapy for bacterial infections. *Front Pharmacol* 2024;15:1513850. <https://doi.org/10.3389/FPHAR.2024.1513850/BIBTEX>.
- [71] Kassab G, Geralde MC, Inada NM, Achilles AE, Guerra VG, Bagnato VS. Nebulization as a tool for photosensitizer delivery to the respiratory tract. *J Biophotonics* 2019;12:e201800189. <https://doi.org/10.1002/JBIO.201800189>.
- [72] Youf R, Nasir A, Müller M, Thétiot F, Haute T, Ghanem R, et al. Ruthenium(II) Polypyridyl Complexes for Antimicrobial Photodynamic Therapy: Prospects for Application in Cystic Fibrosis Lung Airways. *Pharmaceutics* 2022;14. <https://doi.org/10.3390/pharmaceutics14081664>.
- [73] Rahman Sabuj MZ, Islam N. Inhaled antibiotic-loaded polymeric nanoparticles for the management of lower respiratory tract infections. *Nanoscale Adv* 2021;3:4005–18. <https://doi.org/10.1039/D1NA00205H>.
- [74] Lan M, Zhao S, Liu W, Lee CS, Zhang W, Wang P. Photosensitizers for Photodynamic Therapy. *Adv Healthc Mater* 2019;8:1900132. <https://doi.org/10.1002/ADHM.201900132>.
- [75] Ghorbani J, Rahban D, Aghamiri S, Teymouri A, Bahador A. Photosensitizers in antibacterial photodynamic therapy: an overview. *Laser Ther* 2018;27:293. https://doi.org/10.5978/ISLSM.27_18-RA-01.
- [76] Tegos GP, Hamblin MR. Phenothiazinium Antimicrobial Photosensitizers Are Substrates of Bacterial Multidrug Resistance Pumps. *Antimicrob Agents Chemother* 2006;50:196. <https://doi.org/10.1128/AAC.50.1.196-203.2006>.
- [77] Le Gall T, Lemerrier G, Chevreux S, Tücking KS, Ravel J, Thétiot F, et al. Ruthenium(II) Polypyridyl Complexes as Photosensitizers for Antibacterial Photodynamic Therapy: A Structure–Activity Study on Clinical Bacterial Strains. *ChemMedChem* 2018;13:2229–39. <https://doi.org/10.1002/cmdc.201800392>.

- [78] Godard J, Brégier F, Arnoux P, Myrzakhmetov B, Champavier Y, Frochot C, et al. New phenalenone derivatives: Synthesis and evaluation of their singlet oxygen quantum yield. *ACS Omega* 2020;5:28264–72. <https://doi.org/10.1021/acsomega.0c04172>.
- [79] Sandoval-Altamirano C, De la Fuente JR, Berrios E, Sanchez SA, Pizarro N, Morales J, et al. Photophysical characterization of hydroxy and ethoxy phenalenone derivatives. *J Photochem Photobiol A Chem* 2018;353:349–57. <https://doi.org/10.1016/J.JPHOTOCHEM.2017.11.049>.
- [80] Ng XY, Fong KW, Kiew LV, Chung PY, Liew YK, Delsuc N, et al. Ruthenium(II) polypyridyl complexes as emerging photosensitisers for antibacterial photodynamic therapy. *J Inorg Biochem* 2024;250:112425. <https://doi.org/10.1016/J.JINORGBIO.2023.112425>.
- [81] Erdmann E, Villinger A, König B, Seidel WW. 1,10-Phenanthroline-dithiine iridium and ruthenium complexes: Synthesis, characterization and photocatalytic dihydrogen evolution. *Photochemical and Photobiological Sciences* 2018;17:1056–67. <https://doi.org/10.1039/c8pp00068a>.
- [82] Cole HD, Vali A, Roque JA, Shi G, Kaur G, Hodges RO, et al. Ru(II) Phenanthroline-Based Oligothieryl Complexes as Phototherapy Agents. *Inorg Chem* 2023;62:21181–200. <https://doi.org/10.1021/acs.inorgchem.3c03216>.
- [83] Aksakal NE, Kazan HH, Eçik ET, Yuksel F. A novel photosensitizer based on a ruthenium(II) phenanthroline bis(perylene diimide) dyad: synthesis, generation of singlet oxygen and in vitro photodynamic therapy. *New Journal of Chemistry* 2018;42:17538–45. <https://doi.org/10.1039/C8NJ02944J>.
- [84] Youf R, Ghanem R, Nasir A, Lemercier G, Montier T, Le Gall T. Impact of mucus and biofilm on antimicrobial photodynamic therapy: Evaluation using Ruthenium(II) complexes. *Biofilm* 2023;5:100113. <https://doi.org/10.1016/J.BIOFLM.2023.100113>.
- [85] Ye H, Zhou Y, Liu X, Chen Y, Duan S, Zhu R, et al. Recent Advances on Reactive Oxygen Species-Responsive Delivery and Diagnosis System. *Biomacromolecules* 2019;20:2441–63. <https://doi.org/10.1021/acs.biomac.9b00628>.

- [86] Villemain E, Ong YC, Thomas CM, Gasser G. Polymer encapsulation of ruthenium complexes for biological and medicinal applications. *Nat Rev Chem* 2019;3:261–82. <https://doi.org/10.1038/s41570-019-0088-0>.
- [87] Marasini N, Haque S, Kaminskas LM. Polymer-drug conjugates as inhalable drug delivery systems: A review. *Curr Opin Colloid Interface Sci* 2017;31:18–29. <https://doi.org/10.1016/J.COCIS.2017.06.003>.
- [88] Wang Z, Cui F, Sui Y, Yan J. Radical chemistry in polymer science: an overview and recent advances. *Beilstein Journal of Organic Chemistry* 19:116 2023;19:1580–603. <https://doi.org/10.3762/BJOC.19.116>.
- [89] Rimmele M, Glöcklhofer F, Heeney M. Post-polymerisation approaches for the rapid modification of conjugated polymer properties. *Mater Horiz* 2022;9:2678. <https://doi.org/10.1039/D2MH00519K>.
- [90] Kubo T, Easterling CP, Olson RA, Sumerlin BS. Synthesis of multifunctional homopolymers via sequential post-polymerization reactions. *Polym Chem* 2018;9:4605–10. <https://doi.org/10.1039/C8PY01055B>.
- [91] Gao Y, Zhou D, Lyu J, Sigen A, Xu Q, Newland B, et al. Complex polymer architectures through free-radical polymerization of multivinyl monomers. *Nature Reviews Chemistry* 2020 4:4 2020;4:194–212. <https://doi.org/10.1038/s41570-020-0170-7>.
- [92] Saldívar-Guerra E, Vivaldo-Lima E. Handbook of Polymer Synthesis, Characterization, and Processing. Handbook of Polymer Synthesis, Characterization, and Processing 2013. <https://doi.org/10.1002/9781118480793>.
- [93] Braunecker WA, Matyjaszewski K. Controlled/living radical polymerization: Features, developments, and perspectives. *Prog Polym Sci* 2007;32:93–146. <https://doi.org/10.1016/J.PROGPOLYMSCI.2006.11.002>.
- [94] Niu S, Zhang L, Zhu J, Zhang W, Cheng Z, Zhu X. Synthesis of high molecular weight and narrow molecular weight distribution poly(acrylonitrile) via RAFT polymerization. *J Polym Sci A Polym Chem* 2013;51:1197–204. <https://doi.org/10.1002/POLA.26488>.

- [95] Rosenbloom SI, Sifri RJ, Fors BP. Achieving molecular weight distribution shape control and broad dispersities using RAFT polymerizations. *Polym Chem* 2021;12:4910–5. <https://doi.org/10.1039/D1PY00399B>.
- [96] Flynn S, Penrhyn-Lowe OB, Mckeating S, Wright S, Lomas S, Cassin SR, et al. Using temperature to modify the reaction conditions and outcomes of polymers formed using transfer-dominated branching radical telomerisation (TBRT). *RSC Adv* 2022;12:31424–31. <https://doi.org/10.1039/D2RA06578A>.
- [97] Li N, Pan XC. Controlled Radical Polymerization: from Oxygen Inhibition and Tolerance to Oxygen Initiation. *Chinese Journal of Polymer Science (English Edition)* 2021;39:1084–92. <https://doi.org/10.1007/s10118-021-2597-9>.
- [98] Gauthier MA, Gibson MI, Klok HA. Synthesis of Functional Polymers by Post-Polymerization Modification. *Angewandte Chemie International Edition* 2009;48:48–58. <https://doi.org/10.1002/ANIE.200801951>.
- [99] Das A, Theato P. Activated Ester Containing Polymers: Opportunities and Challenges for the Design of Functional Macromolecules. *Chem Rev* 2016;116:1434–95. <https://doi.org/10.1021/acs.chemrev.5b00291>.
- [100] Theato P. Synthesis of well-defined polymeric activated esters. *J Polym Sci A Polym Chem* 2008;46:6677–87. <https://doi.org/10.1002/POLA.22994>.
- [101] Tanaka T. Recent Advances in Polymers Bearing Activated Esters for the Synthesis of Glycopolymers by Postpolymerization Modification. *Polymers* 2024, Vol 16, Page 1100 2024;16:1100. <https://doi.org/10.3390/POLYM16081100>.
- [102] Hansen RA, Märcher A, Gothelf K V. One-Step Conversion of NHS Esters to Reagents for Site-Directed Labeling of IgG Antibodies. *Bioconj Chem* 2022;33:1811–7. <https://doi.org/10.1021/acs.bioconjchem.2c00392>.
- [103] Graisuwan W, Zhao H, Kiatkamjornwong S, Theato P, Hoven VP. Formation of thermo-sensitive and cross-linkable micelles by self-assembly of poly(pentafluorophenyl acrylate)-containing block copolymer. *J Polym Sci A Polym Chem* 2015;53:1103–13. <https://doi.org/10.1002/POLA.27541>.

- [104] Namivandi-Zangeneh R, Kwan RJ, Nguyen TK, Yeow J, Byrne FL, Oehlers SH, et al. The effects of polymer topology and chain length on the antimicrobial activity and hemocompatibility of amphiphilic ternary copolymers. *Polym Chem* 2018;9:1735–44. <https://doi.org/10.1039/C7PY01069A>.
- [105] Pearce AK, O'Reilly RK. Polymers for Biomedical Applications: The Importance of Hydrophobicity in Directing Biological Interactions and Application Efficacy. *Biomacromolecules*, 2021;22:4459–69. <https://doi.org/10.1021/acs.biomac.1c00434>.
- [106] Park K, Kim JH, Nam YS, Lee S, Nam HY, Kim K, et al. Effect of polymer molecular weight on the tumor targeting characteristics of self-assembled glycol chitosan nanoparticles. *Journal of Controlled Release* 2007;122:305–14. <https://doi.org/10.1016/J.JCONREL.2007.04.009>.
- [107] Zhao C, Li X, Li L, Cheng G, Gong X, Zheng J. Dual functionality of antimicrobial and antifouling of poly(n -hydroxyethylacrylamide)/salicylate hydrogels. *Langmuir* 2013;29:1517–24. https://doi.org/10.1021/LA304511S/ASSET/IMAGES/LARGE/LA-2012-04511S_0011.JPEG.
- [108] Yang J, Zhang M, Chen H, Chang Y, Chen Z, Zheng J. Probing the structural dependence of carbon space lengths of poly(N -hydroxyalkyl acrylamide)-based brushes on antifouling performance. *Biomacromolecules* 2014;15:2982–91. https://doi.org/10.1021/BM500598A/SUPPL_FILE/BM500598A_SI_001.PDF.
- [109] Lim YH, Tiemann KM, Hunstad DA, Elsabahy M, Wooley KL. Polymeric Nanoparticles in Development for Treatment of Pulmonary Infectious Diseases. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 2016;8:842. <https://doi.org/10.1002/WNAN.1401>.
- [110] Zhang Y, Sun C. Current status, challenges and prospects of antifouling materials for oncology applications. *Front Oncol* 2024;14:1391293. <https://doi.org/10.3389/FONC.2024.1391293/BIBTEX>.
- [111] Henning A, Schneider M, Nafee N, Muijs L, Rytting E, Wang X, et al. Influence of Particle Size and Material Properties on Mucociliary Clearance from the

- Airways. <https://HomeLiebertpubCom/Jamp> 2010;23:233–41.
<https://doi.org/10.1089/JAMP.2009.0806>.
- [112] Galstyan A, Stokov K. Influence of photosensitizer concentration and polymer composition on photoinduced antimicrobial activity of PVA- and PVA-chitosan-based electrospun nanomaterials cross-linked with tailor-made silicon(IV) phthalocyanine. *Photochemical and Photobiological Sciences* 2022;21:1387–98. <https://doi.org/10.1007/S43630-022-00229-9/FIGURES/5>.
- [113] Banerjee A, Chowdhury P, Bauri K, Saha B, De P. Inhibition and eradication of bacterial biofilm using polymeric materials. *Biomater Sci* 2022;11:11–36. <https://doi.org/10.1039/D2BM01276F>.
- [114] Dotsenko IA, Curtis M, Samoshina NM, Samoshin V V. Convenient synthesis of 5-aryl(alkyl)sulfanyl-1,10-phenanthrolines from 5,6-epoxy-5,6-dihydro-1,10-phenanthroline, and their activity towards fungal β -d-glycosidases. *Tetrahedron* 2011;67:7470–8. <https://doi.org/10.1016/j.tet.2011.07.058>.
- [115] Rousset E, Mongin O, Moreau J, Lawson-Daku LM, Beley M, Gros PC, et al. Molecular engineering for optical properties of 5-substituted-1,10-phenanthroline-based Ru(II) complexes. *Dalton Transactions* 2021;50:10119–32. <https://doi.org/10.1039/d1dt00886b>.
- [116] Mongin O, Four M, Chevreux S, Blanchard-Desce M, Lemerrier G. Two-photon absorption engineering of 5-(Fluorenyl)-1,10-phenanthroline-based Ru(II) Complexes. *Chimia (Aarau)* 2015;69:666–9. <https://doi.org/10.2533/chimia.2015.666>.
- [117] Eberhardt M, Mruk R, Zentel R, Théato P. Synthesis of pentafluorophenyl(meth)acrylate polymers: New precursor polymers for the synthesis of multifunctional materials. *Eur Polym J* 2005;41:1569–75. <https://doi.org/10.1016/j.eurpolymj.2005.01.025>.
- [118] Nilles K, Theato P. Synthesis and polymerization of active ester monomers based on 4-vinylbenzoic acid. *Eur Polym J* 2007;43:2901–12. <https://doi.org/10.1016/j.eurpolymj.2007.04.038>.

- [119] Ru J-X, Guan L-P, Tang X-L, Dou W, Yao X, Chen W-M, et al. Turn-on Phosphorescent Chemodosimeter for Hg 2+ Based on a Cyclometalated Ir(III) Complex and Its Application in Time-Resolved Luminescence Assays and Live Cell Imaging 2014. <https://doi.org/10.1021/ic501417s>.
- [120] Villien M, Deroo S, Gicquel E, Defrancq E, Moucheron C, Kirsch-De Mesmaeker A, et al. The oxime bond formation as an efficient tool for the conjugation of ruthenium complexes to oligonucleotides and peptides. *Tetrahedron* 2007;63:11299–306. <https://doi.org/10.1016/j.tet.2007.08.088>.
- [121] Bellam R, Jaganyi D, Mambanda A, Robinson R. Role of a 2,3-bis(pyridyl)pyrazinyl chelate bridging ligand in the reactivity of Ru(ii)-Pt(ii) dinuclear complexes on the substitution of chlorides by thiourea nucleophiles-a kinetic study. *New Journal of Chemistry* 2018;42:12557–69. <https://doi.org/10.1039/c8nj02096e>.
- [122] Xu Q, Feng H, Shao X. Light-induced activities of novel naphtho[1,8-ef]isoindole-7,8,10(9H)-trione and oxoisoaporphine derivatives towards mosquito larvae. *Bioorg Med Chem Lett* 2021;48:128225. <https://doi.org/10.1016/J.BMCL.2021.128225>.
- [123] Song R, Feng Y, Wang D, Xu Z, Li Z, Shao X. Phytoalexin Phenalenone Derivatives Inactivate Mosquito Larvae and Root-knot Nematode as Type-II Photosensitizer. *Scientific Reports* 2017 7:1 2017;7:1–9. <https://doi.org/10.1038/srep42058>.
- [124] Späth A, Leibl C, Cieplik F, Lehner K, Regensburger J, Hiller KA, et al. Improving photodynamic inactivation of bacteria in dentistry: Highly effective and fast killing of oral key pathogens with novel tooth-colored type-II photosensitizers. *J Med Chem*,2014;57:5157–68. <https://doi.org/10.1021/JM4019492>.
- [125] L. F. Fieser, E. B. Hershberg, *J. Am. Chem. Soc.* 1938, 60, 1658.
- [126] A. Späth, A. Eichner, Phenalen-1-on-haltige Photosensibilisator-zusammensetzung, Phenalen-1-on-verbindungen sowie deren Verwendung, 2018, EP 3 375 7 74 A1 Patent n.d.

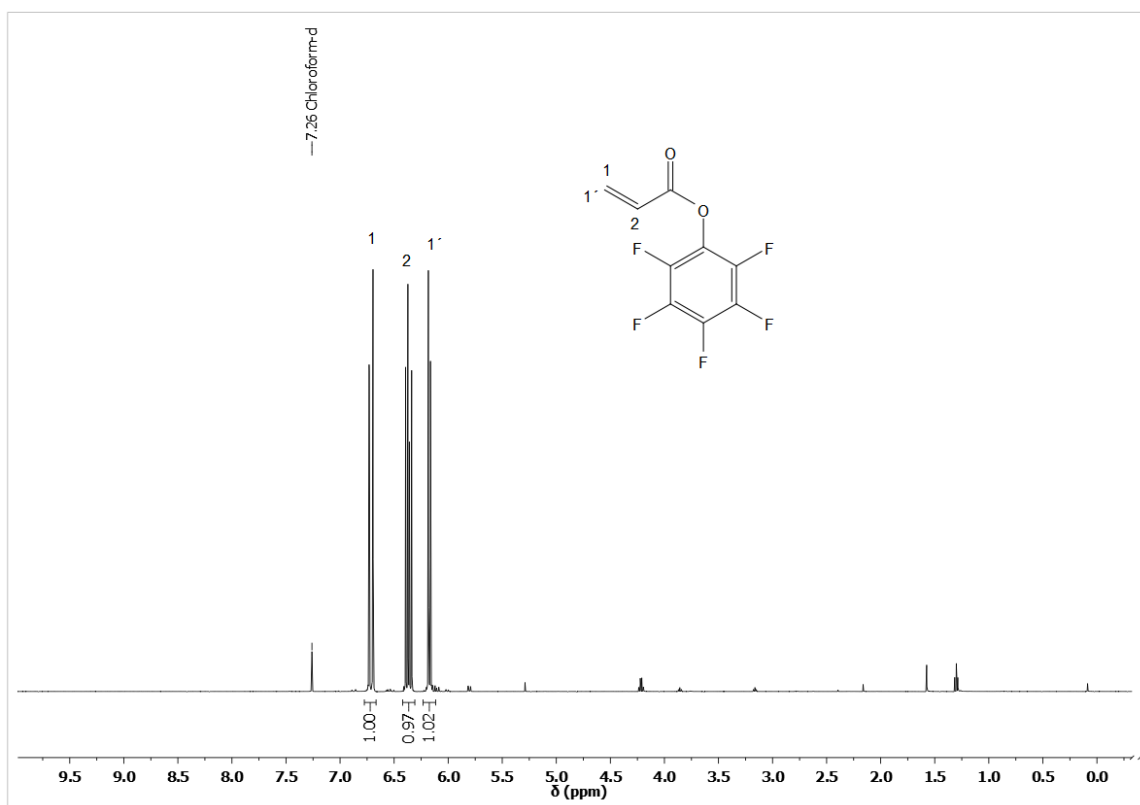
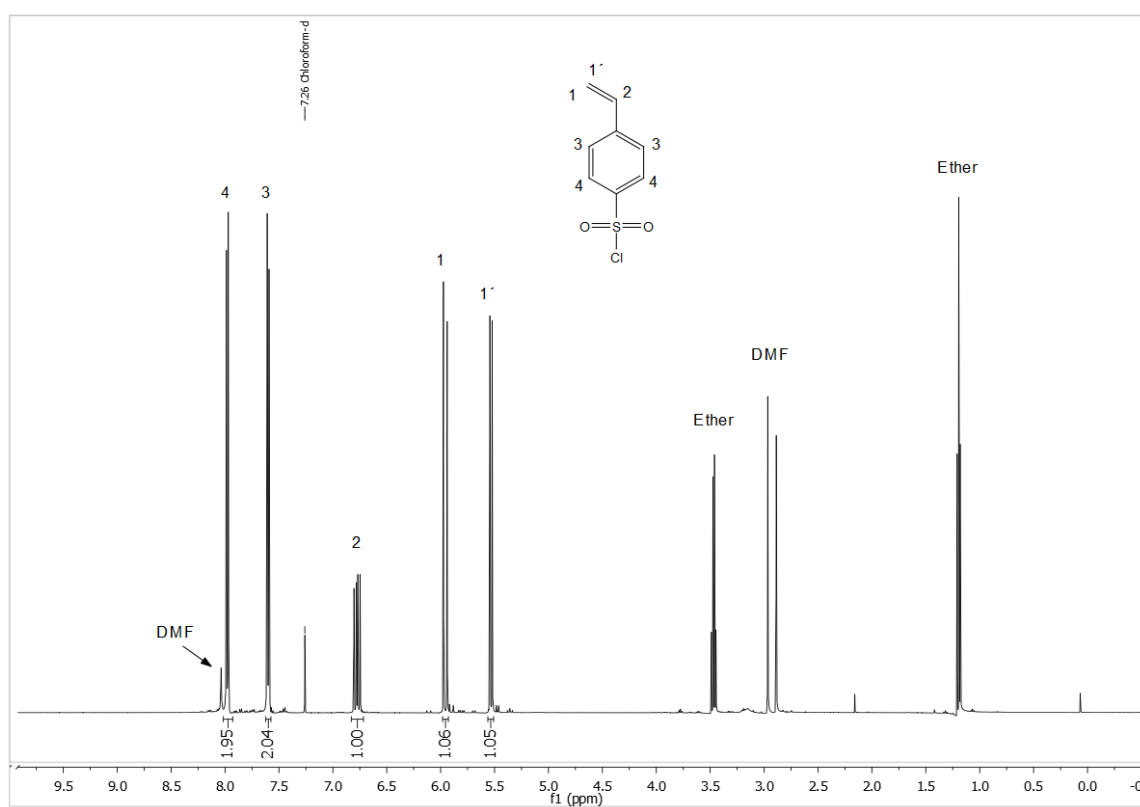
- [127] Pittelkow M, Christensen JB. Convergent synthesis of internally branched PAMAM dendrimers. *Org Lett* 2005;7:1295–8.
https://doi.org/10.1021/OL050040D/SUPPL_FILE/OL050040DSI20050126_074716.
- [128] Guo S, Huang Q, Chen Y, Wei J, Zheng J, Wang L, et al. Synthesis and Bioactivity of Guanidinium-Functionalized Pillar[5]arene as a Biofilm Disruptor. *Angewandte Chemie International Edition* 2021;60:618–23.
<https://doi.org/10.1002/ANIE.202013975>.
- [129] Samrot A V., Mohamed AA, Faradjeva E, Jie LS, Sze CH, Arif A, et al. Mechanisms and Impact of Biofilms and Targeting of Biofilms Using Bioactive Compounds—A Review. *Medicina (B Aires)* 2021;57:839.
<https://doi.org/10.3390/MEDICINA57080839>.
- [130] Alves FRF, Silva MG, Rôç IN, Siqueira JF. Biofilm biomass disruption by natural substances with potential for endodontic use. *Braz Oral Res* 2013;27:20–5. <https://doi.org/10.1590/S1806-83242013000100004>.
- [131] Böttcher T, Kolodkin-Gal I, Kolter R, Losick R, Clardy J. Synthesis and activity of biomimetic biofilm disruptors. *J Am Chem Soc* 2013;135:2927–30.
https://doi.org/10.1021/JA3120955/SUPPL_FILE/JA3120955_SI_001.PDF.
- [132] Pantos A, Tsogas I, Paleos CM. Guanidinium group: A versatile moiety inducing transport and multicompartmentalization in complementary membranes. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 2008;1778:811–23. <https://doi.org/10.1016/J.BBAMEM.2007.12.003>.
- [133] Alves FRF, Silva MG, Rôç IN, Siqueira JF. Biofilm biomass disruption by natural substances with potential for endodontic use. *Braz Oral Res* 2013;27:20–5. <https://doi.org/10.1590/S1806-83242013000100004>.
- [134] Saptami K, Arokia Balaya Rex D, Chandrasekaran J, Rekha PD. Competitive interaction of thymol with cviR inhibits quorum sensing and associated biofilm formation in *Chromobacterium violaceum*. *International Microbiology* 2022;25:629–38. <https://doi.org/10.1007/S10123-022-00247-8/FIGURES/6>.

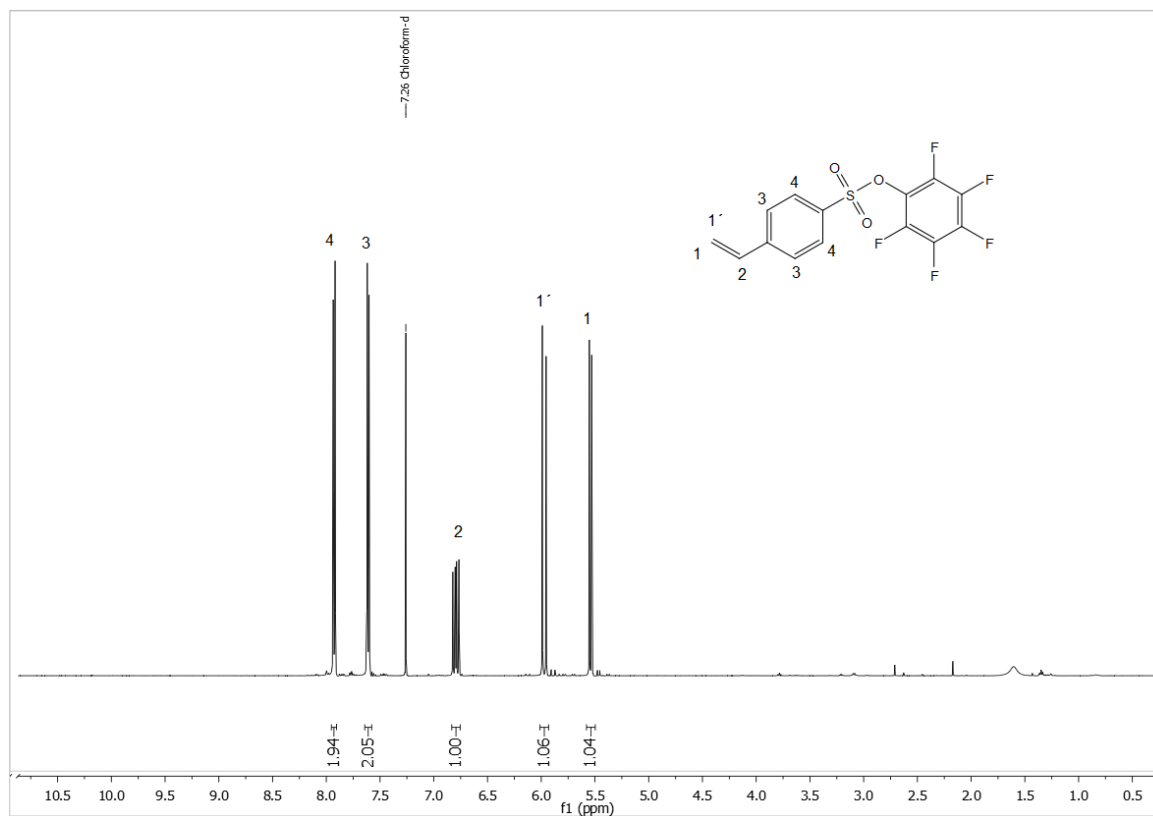
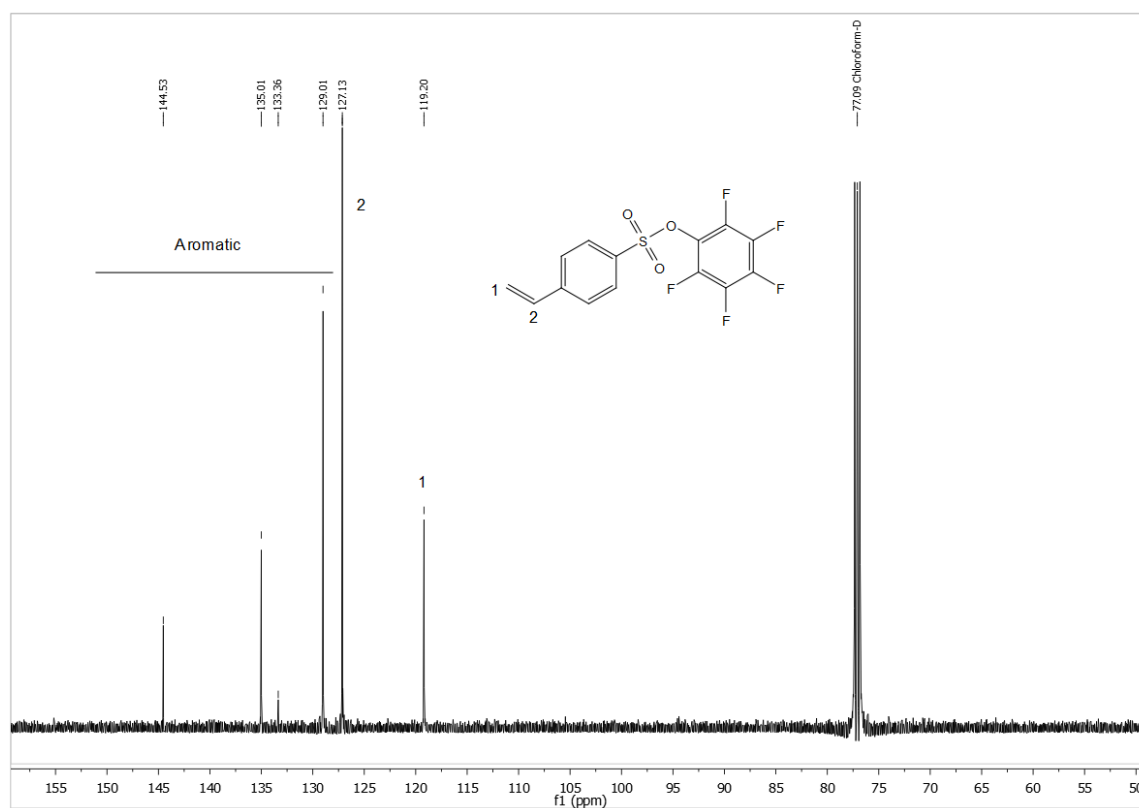
- [135] Liu Q, Niu H, Zhang W, Mu H, Sun C, Duan J. Synergy among thymol, eugenol, berberine, cinnamaldehyde and streptomycin against planktonic and biofilm-associated food-borne pathogens. *Lett Appl Microbiol* 2015;60:421–30. <https://doi.org/10.1111/LAM.12401>.
- [136] Nostro A, Roccaro AS, Bisignano G, Marino A, Cannatelli MA, Pizzimenti FC, et al. Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. *J Med Microbiol* 2007;56:519–23. <https://doi.org/10.1099/JMM.0.46804-0/CITE/REFWORKS>.
- [137] Freese S, Diraoui S, Mateescu A, Frank P, Theodorakopoulos C, Jonas U. Polyolefin-supported hydrogels for selective cleaning treatments of paintings. *Gels* 2020;6. <https://doi.org/10.3390/gels6010001>.
- [138] Kirchner S, Fothergill JL, Wright EA, James CE, Mowat E, Winstanley C. Use of artificial sputum medium to test antibiotic efficacy against *Pseudomonas aeruginosa* in conditions more relevant to the cystic fibrosis lung. *Journal of Visualized Experiments* 2012:1–8. <https://doi.org/10.3791/3857>.
- [139] Discekici EH, Shankel SL, Anastasaki A, Oschmann B, Lee IH, Niu J, et al. Dual-pathway chain-end modification of RAFT polymers using visible light and metal-free conditions. *Chem Commun (Camb)* 2017;53:1888. <https://doi.org/10.1039/C6CC08370F>.
- [140] Baba T, Bae T, Schneewind O, Takeuchi F, Hiramatsu K. Genome sequence of *Staphylococcus aureus* strain newman and comparative analysis of staphylococcal genomes: Polymorphism and evolution of two major pathogenicity islands. *J Bacteriol* 2008;190:300–10. <https://doi.org/10.1128/JB.01000-07>.
- [141] Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, et al. Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *Lancet* 2001;357:1225–40. [https://doi.org/10.1016/S0140-6736\(00\)04403-2](https://doi.org/10.1016/S0140-6736(00)04403-2).
- [142] BACTOME START PAGE n.d. <https://bactome.helmholtz-hzi.de/cgi-bin/h-intro.cgi> (accessed February 26, 2025).

- [143] Kaur K, Müller M, Müller M, Schönherr H. Photodynamic Eradication of *Pseudomonas aeruginosa* with Ru-Photosensitizers Encapsulated in Enzyme Degradable Nanocarriers. *Pharmaceutics* 2023;15:2683. <https://doi.org/10.3390/PHARMACEUTICS15122683/S1>.
- [144] Baba T, Bae T, Schneewind O, Takeuchi F, Hiramatsu K. Genome sequence of *Staphylococcus aureus* strain newman and comparative analysis of staphylococcal genomes: Polymorphism and evolution of two major pathogenicity islands. *J Bacteriol* 2008;190:300–10. <https://doi.org/10.1128/JB.01000-07/ASSET/F738BAF0-382D-4C24-B27F-76025BB74EE8/ASSETS/GRAPHIC/ZJB0010874080004.JPEG>.
- [145] Seyler TM, Moore C, Kim H, Ramachandran S, Agris PF. A New Promising Anti-Infective Agent Inhibits Biofilm Growth by Targeting Simultaneously a Conserved RNA Function That Controls Multiple Genes. *Antibiotics* 2021;10:41. <https://doi.org/10.3390/ANTIBIOTICS10010041>.
- [146] Stojowska-Swędryńska K, Kuczyńska-Wiśnik D, Laskowska E. New Strategies to Kill Metabolically-Dormant Cells Directly Bypassing the Need for Active Cellular Processes. *Antibiotics* 2023;12:1044. <https://doi.org/10.3390/ANTIBIOTICS12061044>.
- [147] Brown HL, van Vliet AHM, Betts RP, Reuter M. Tetrazolium reduction allows assessment of biofilm formation by *Campylobacter jejuni* in a food matrix model. *J Appl Microbiol* 2013;115:1212–21. <https://doi.org/10.1111/JAM.12316>.
- [148] Jochum FD, Theato P. Temperature- and light-responsive polyacrylamides prepared by a double polymer analogous reaction of activated ester polymers. *Macromolecules* 2009;42:5941–5. <https://doi.org/10.1021/ma900945s>.
- [149] Nilles K, Theato P. Polymerization of an activated ester monomer based on 4-vinylsulfonic acid and its polymer analogous reaction † ‡ n.d. <https://doi.org/10.1039/c0py00261e>.
- [150] Starling J, Da Silva CM, Silva Dantas MS, De Fátima Â, Oréfice RL. N-acryloxysuccinimide: Synthesis, characterization, and incorporation in dental adhesives. *Int J Adhes Adhes* 2011;31:767–74. <https://doi.org/10.1016/J.IJADHADH.2011.07.006>.

- [151] Xavier FJS, Rodrigues KA da F, de Oliveira RG, Lima Junior CG, Rocha J da C, Keesen TSL, et al. Synthesis and In Vitro Anti Leishmania amazonensis Biological Screening of Morita-Baylis-Hillman Adducts Prepared from Eugenol, Thymol and Carvacrol. *Molecules* 2016, Vol 21, Page 1483 2016;21:1483. <https://doi.org/10.3390/MOLECULES21111483>.
- [152] Das A, Theato P. Multifaceted Synthetic Route to Functional Polyacrylates by Transesterification of Poly(pentafluorophenyl acrylates). *Macromolecules* 2015;48:8695–707. <https://doi.org/10.1021/acs.macromol.5b02293>.
- [153] Shamout F, Monaco A, Yilmaz G, Remzi Becer C, Hartmann L et al. Synthesis of Brush-Like Glycopolymers with Monodisperse, Sequence-Defined Side Chains and Their Interactions with Plant and Animal Lectins. *Macromol Rapid Commun* 2020;41:1900459. <https://doi.org/10.1002/MARC.201900459>.

7 Appendix

Fig. SI 1: ^1H NMR of PFPA (21).Fig. SI 2: ^1H NMR of 4-vinylbenzene-1-sulfonyl chloride.

Fig. SI 3: ¹H NMR of pentafluorophenyl 4-vinylbenzene sulfonate (PFP-4-VBS) (22)Fig. SI 4: ¹³C NMR of pentafluorophenyl 4-vinylbenzene sulfonate (PFP-4-VBS) (22)

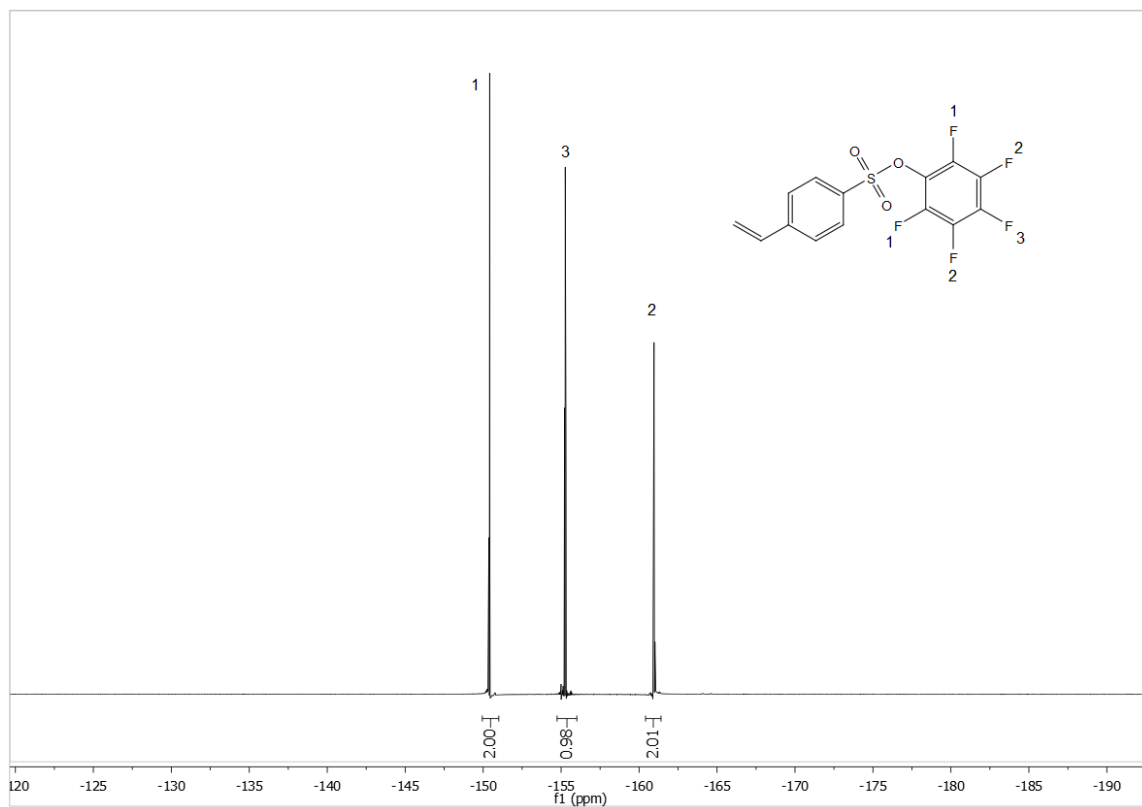


Fig. SI 5: ^{19}F NMR of pentafluorophenyl 4-vinylbenzene sulfonate (PFP-4-VBS) (22).

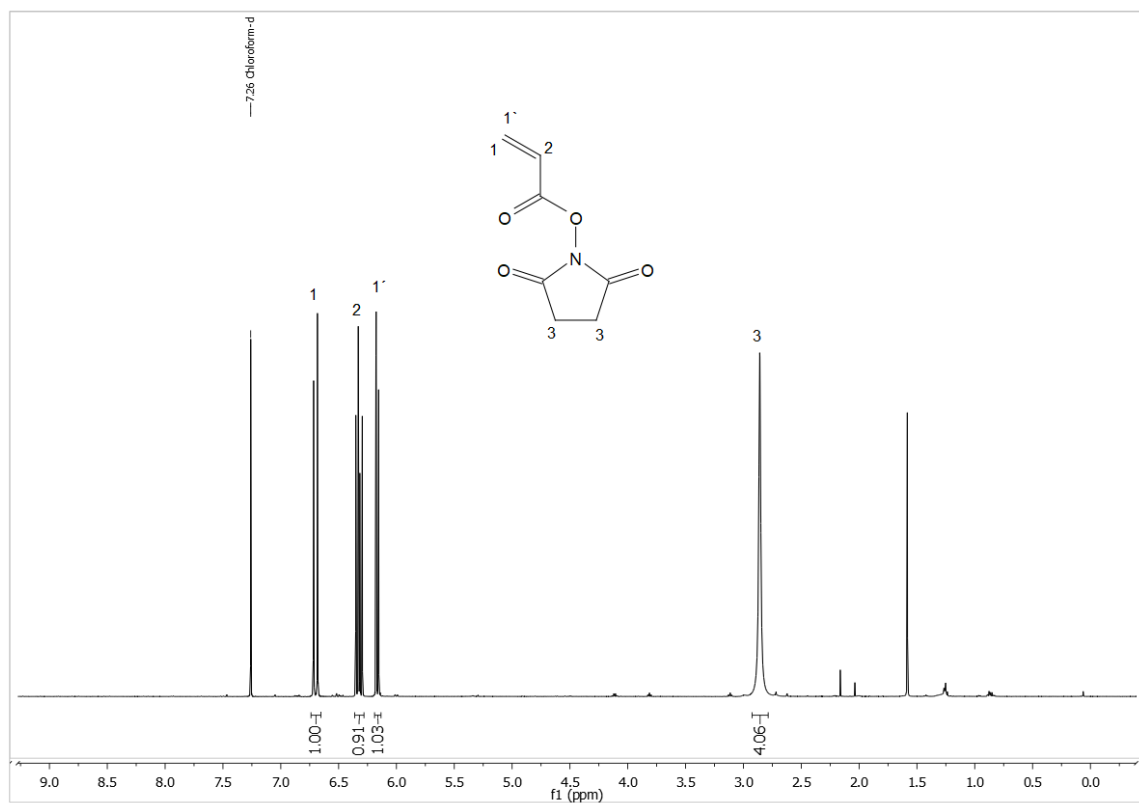
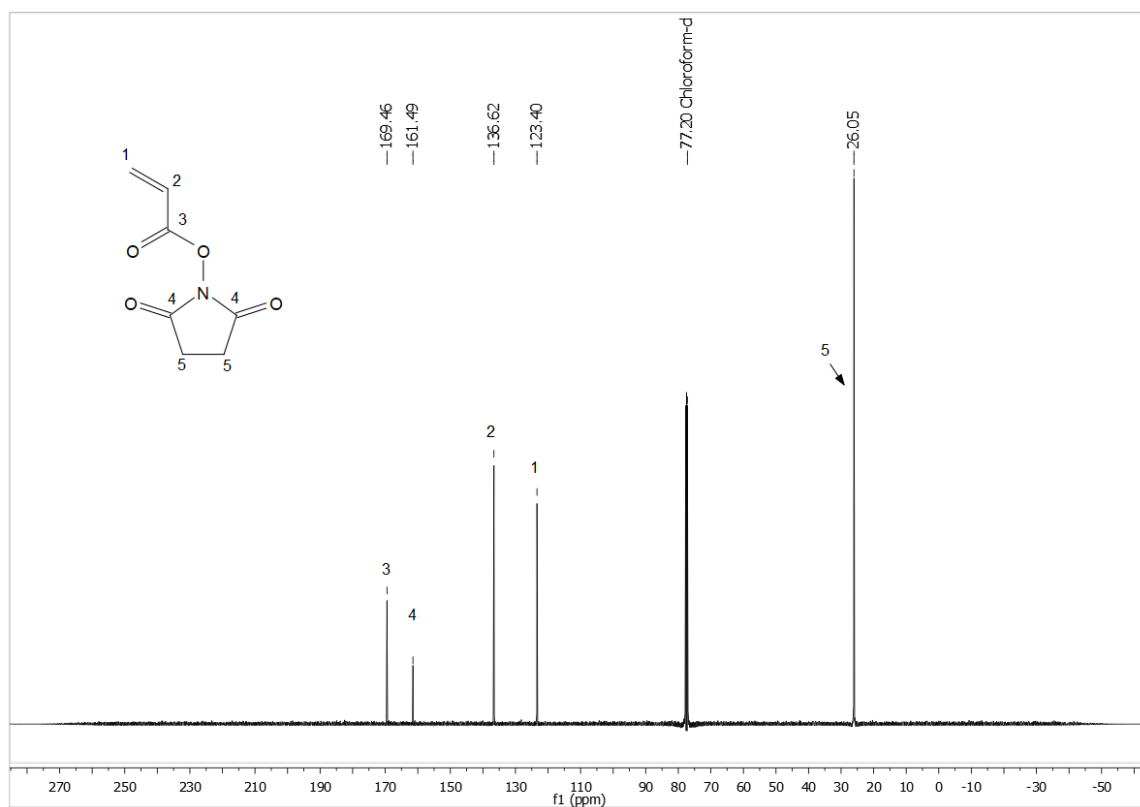
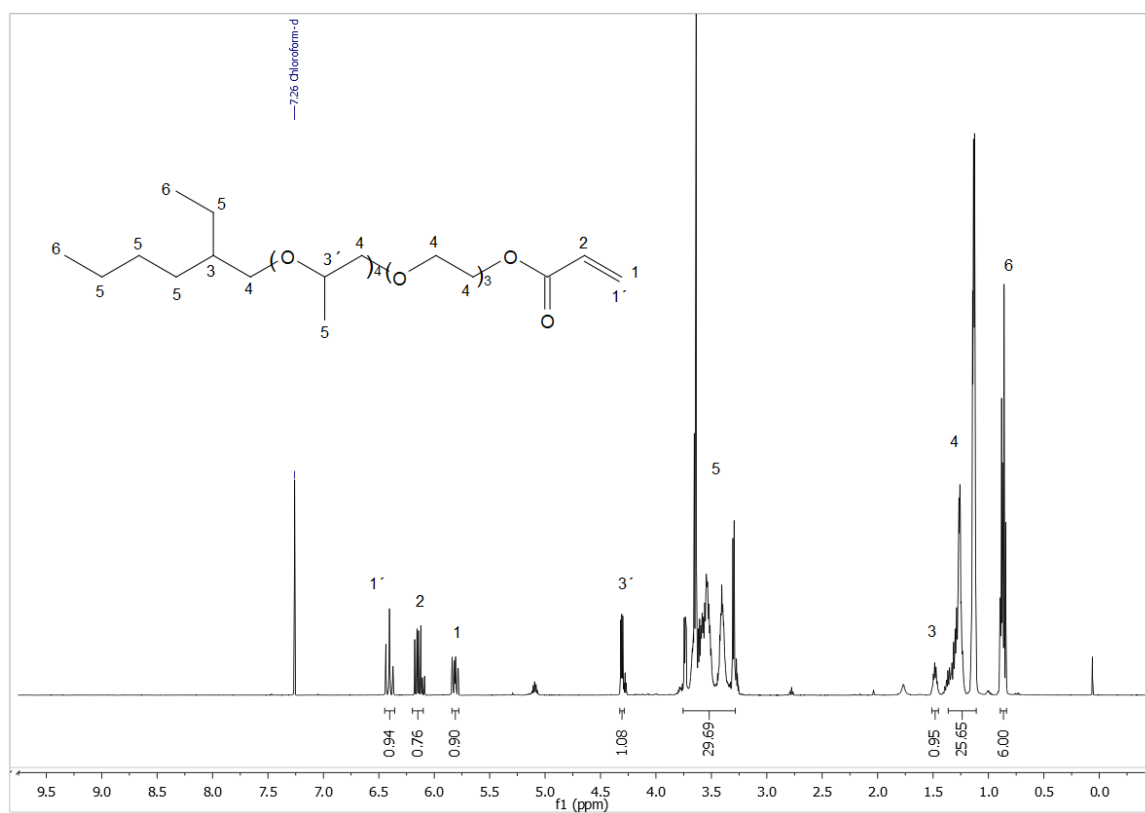


Fig. SI 6: ^1H NMR of N-hydroxysuccinimide acrylate (NHSA) (23).

Fig. SI 7: ^{13}C NMR of N-hydroxysuccinimide acrylate (NHSA) (23)Fig. SI 8: ^1H NMR of Ecosurf acrylate EH3A (24 or SurfEH3A).

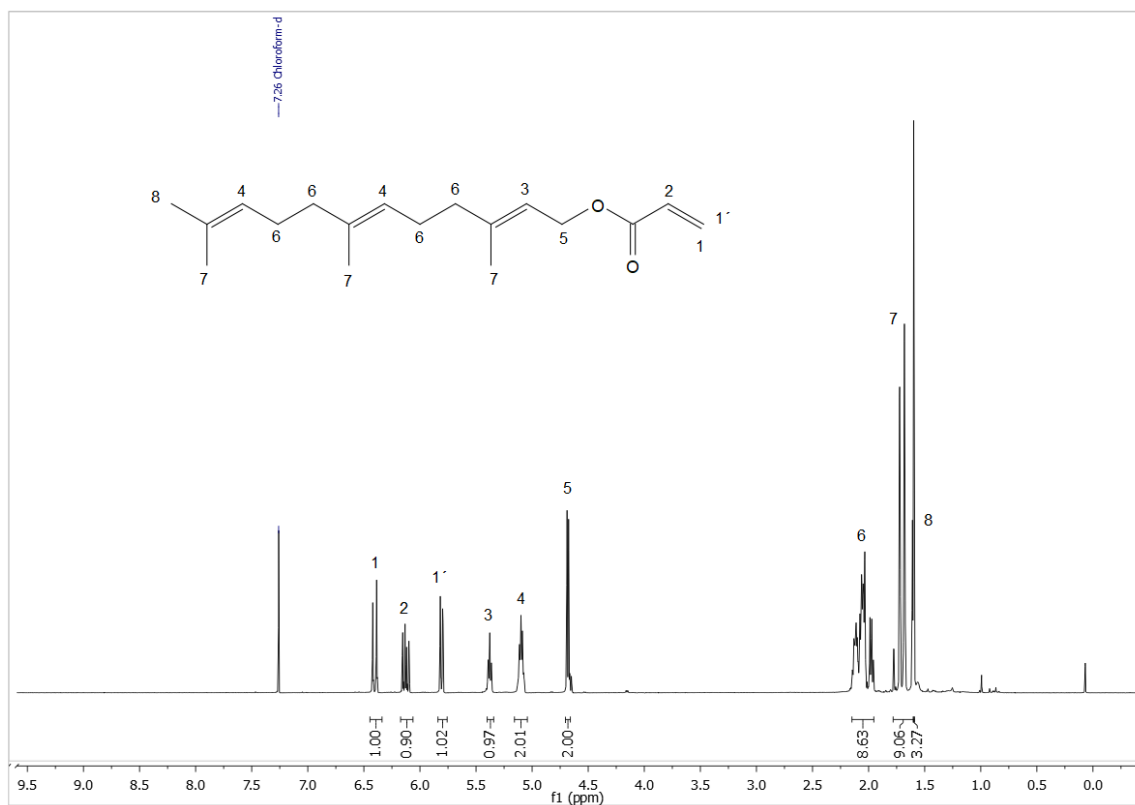


Fig. SI 9: ¹H NMR of (2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl acrylate (Farnesol acrylate) (19).

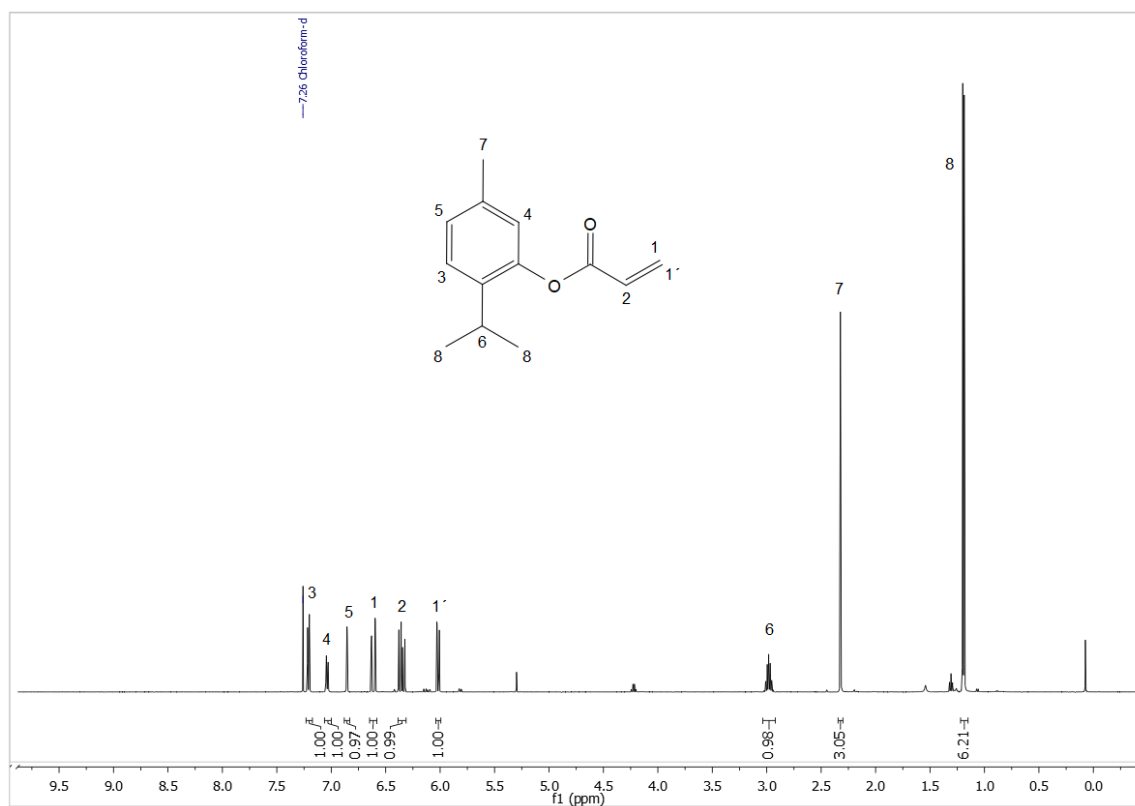
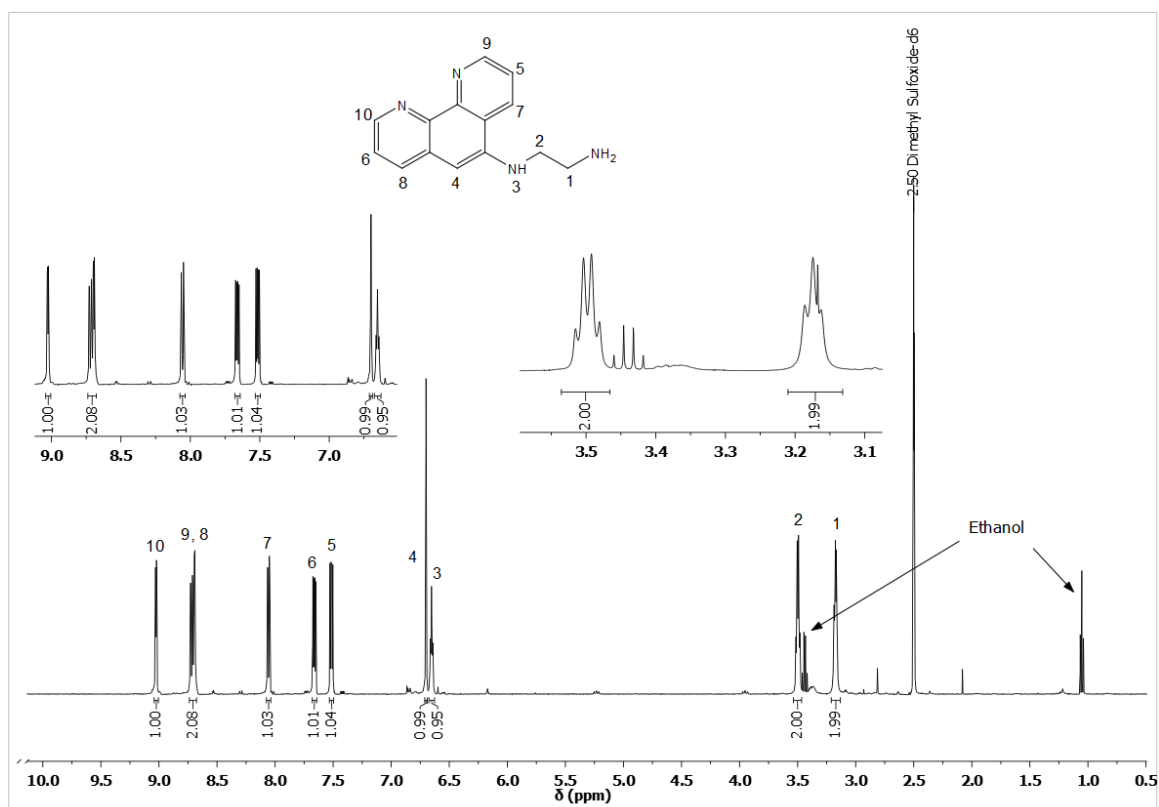
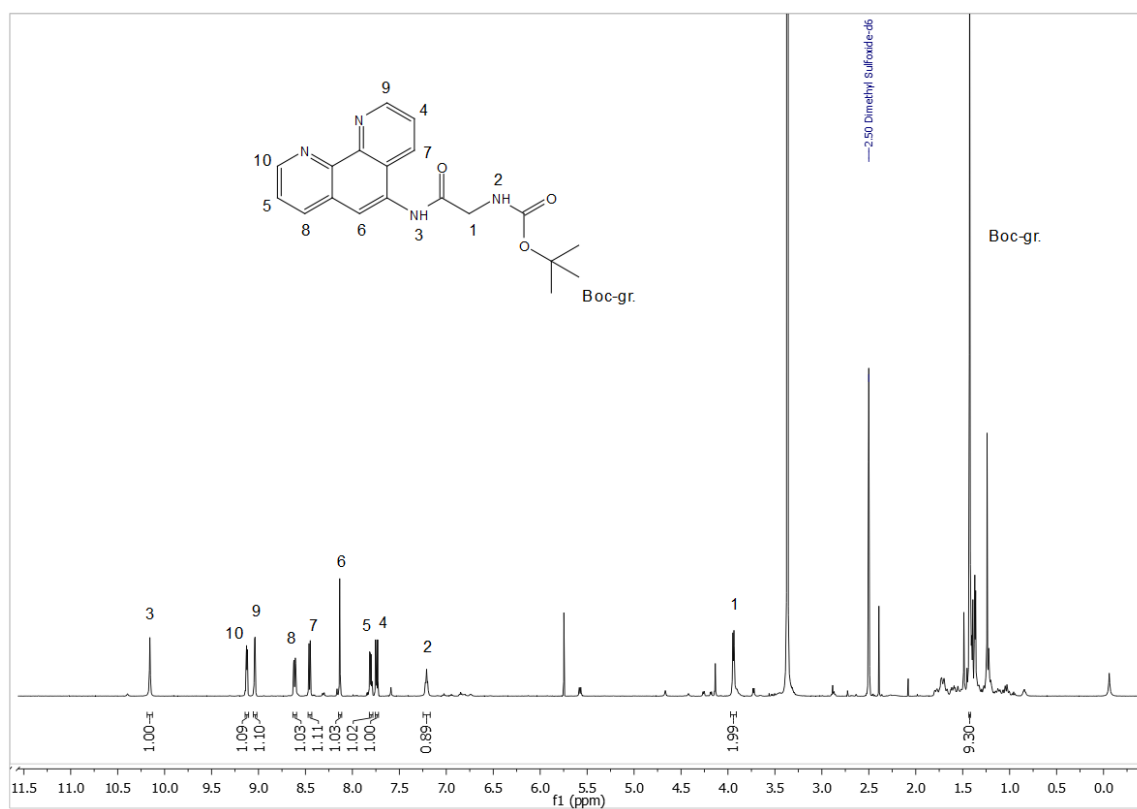
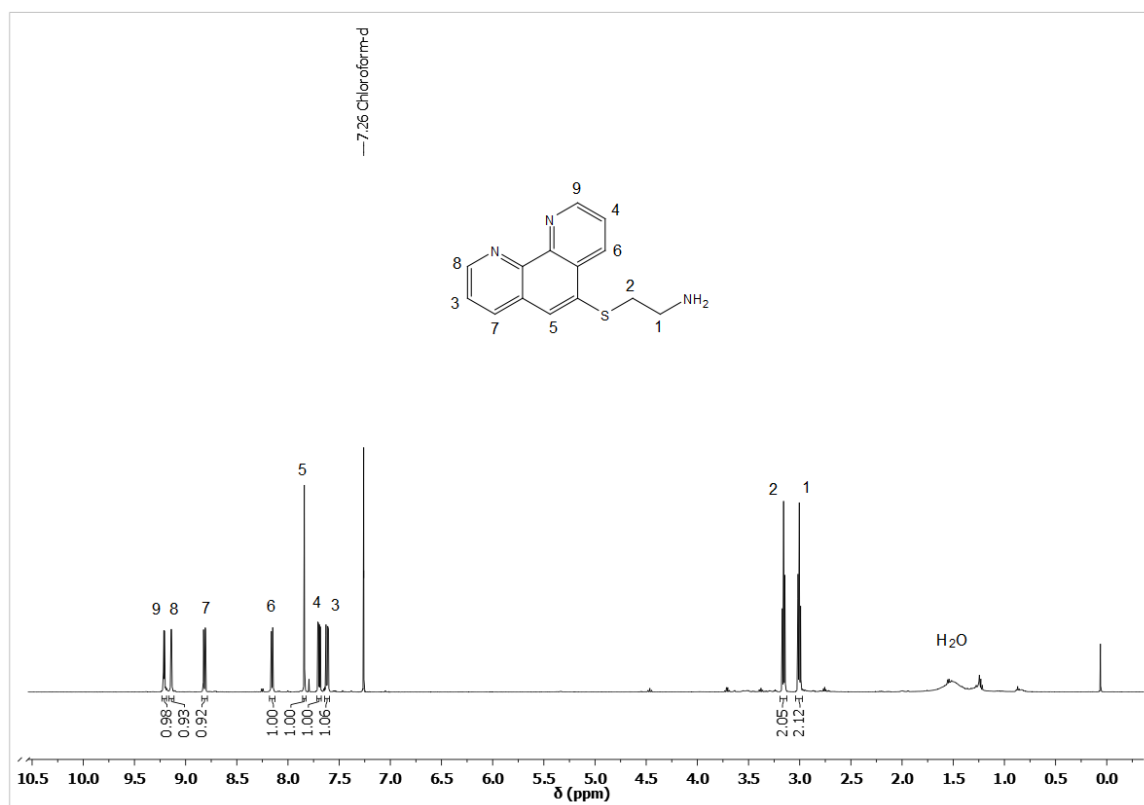
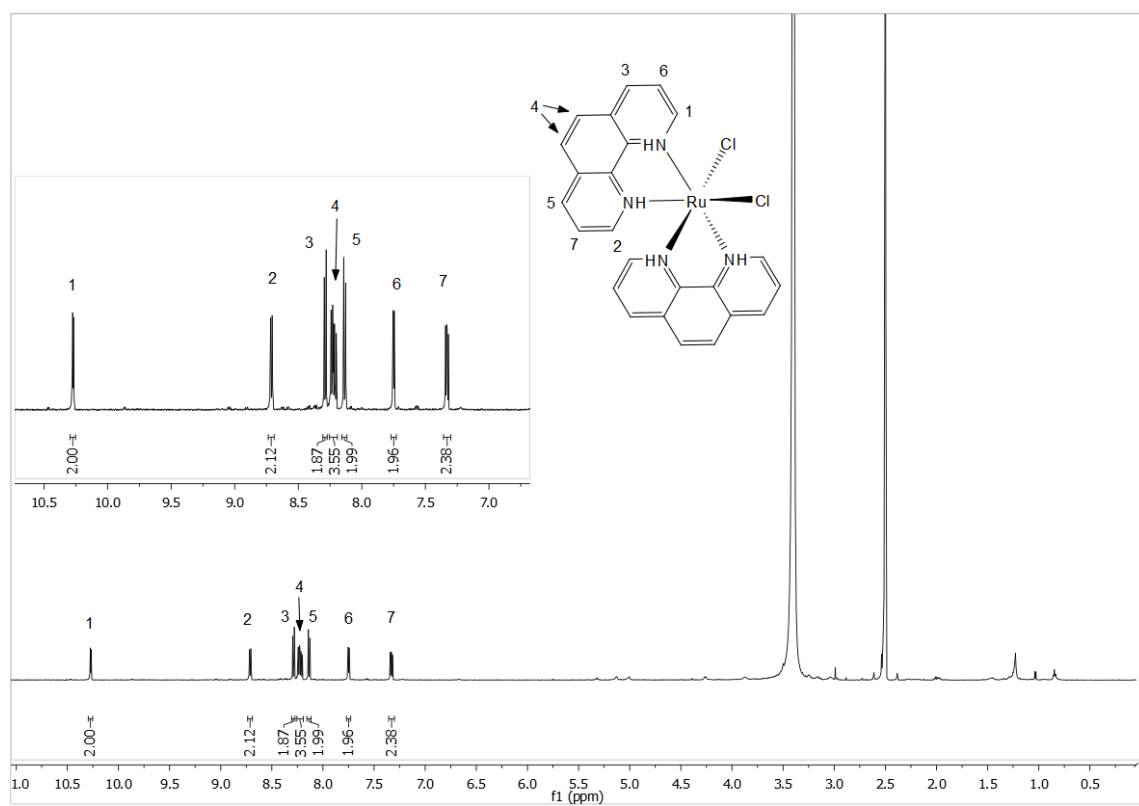
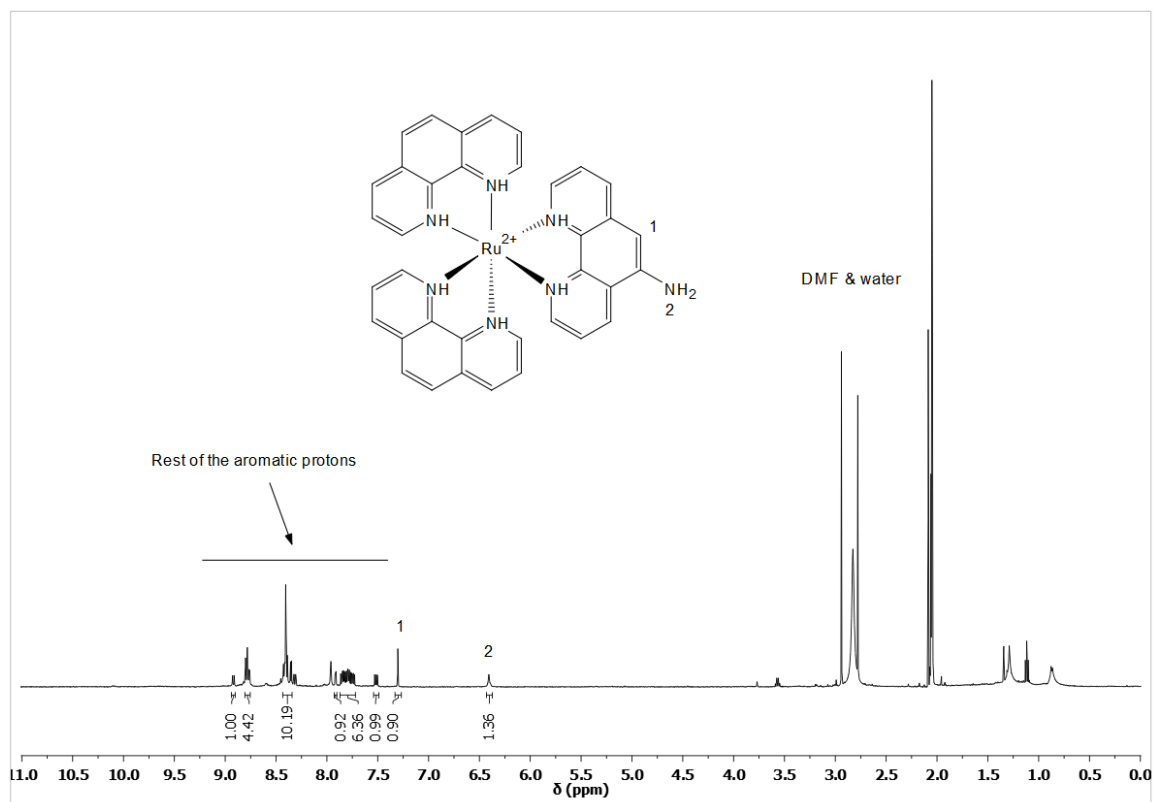
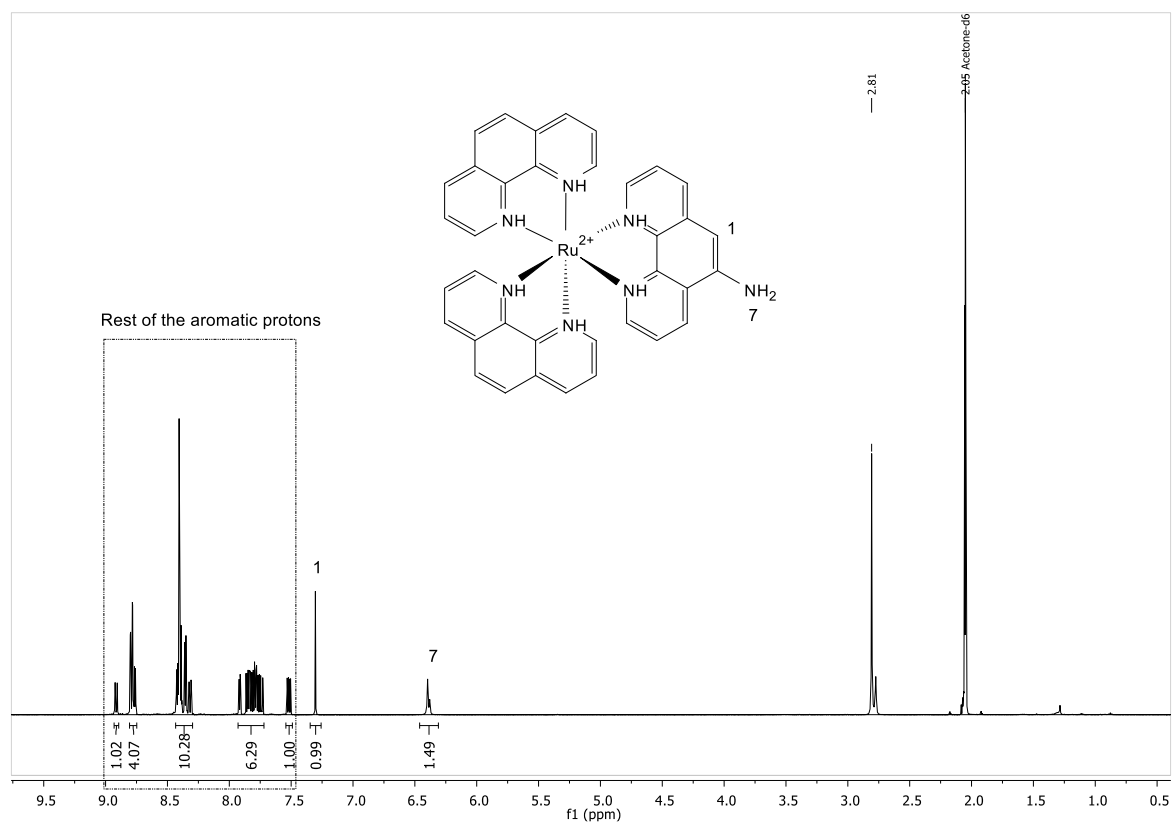
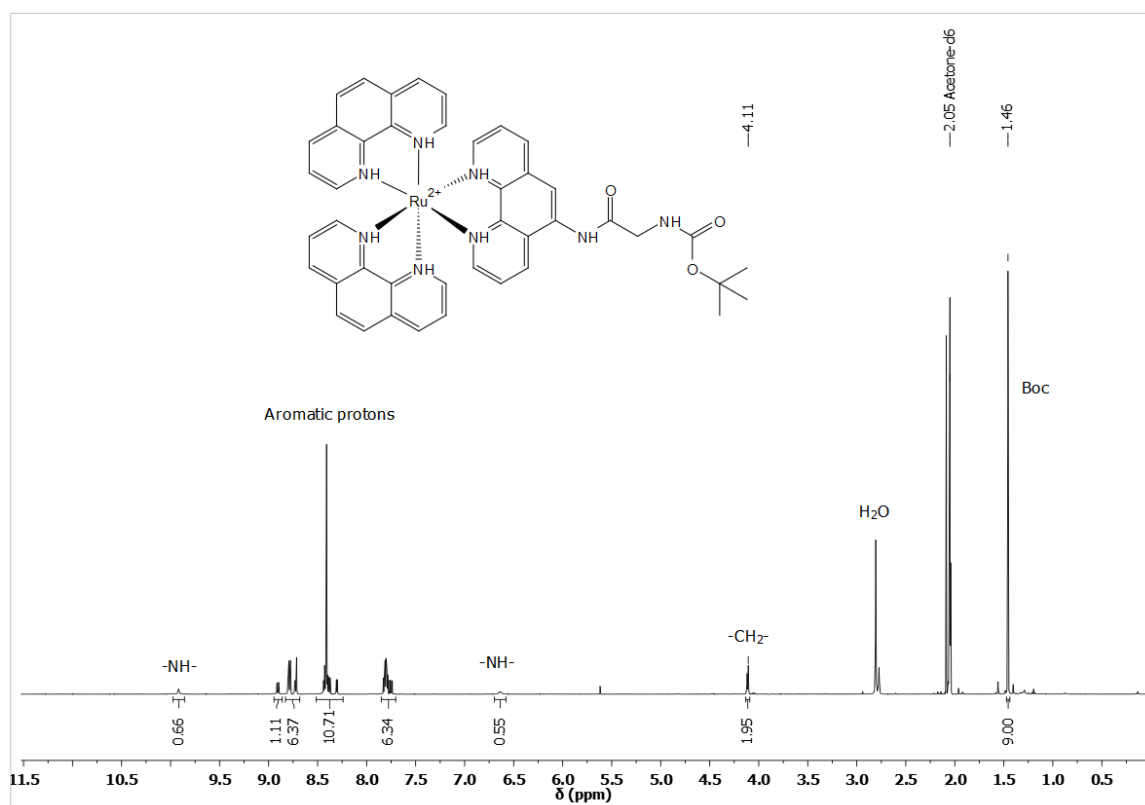
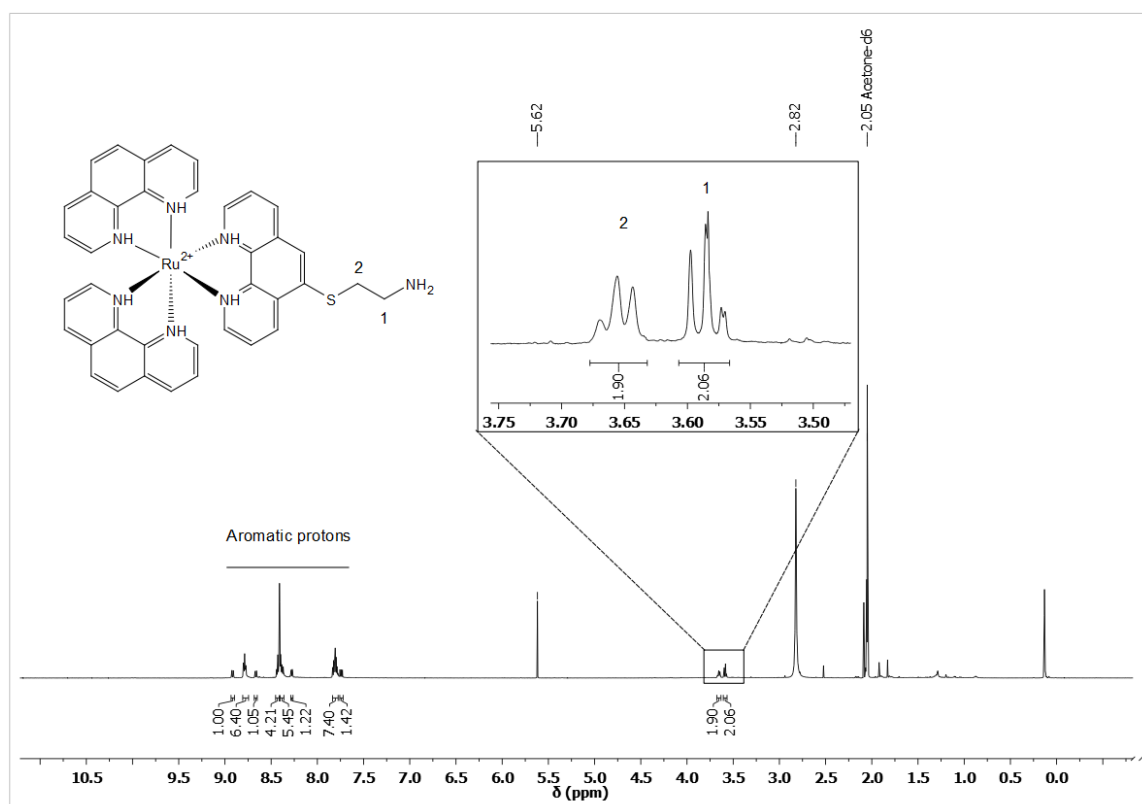


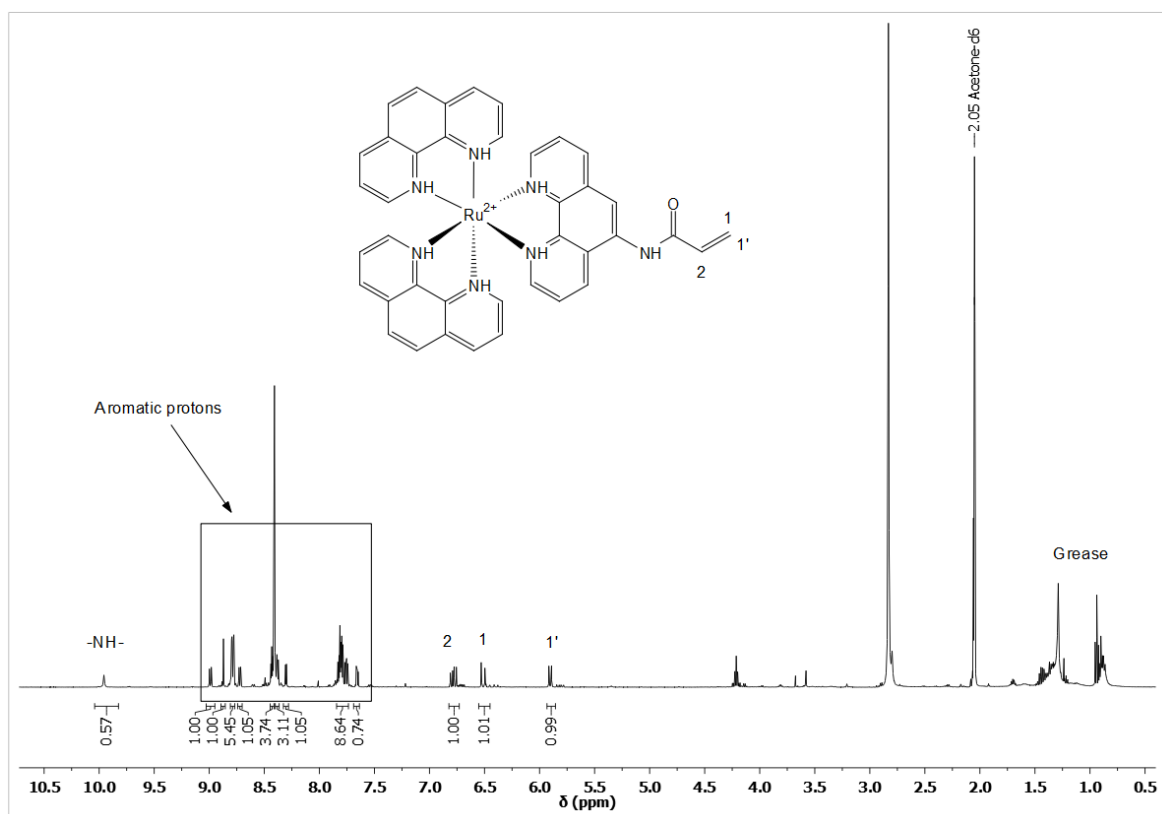
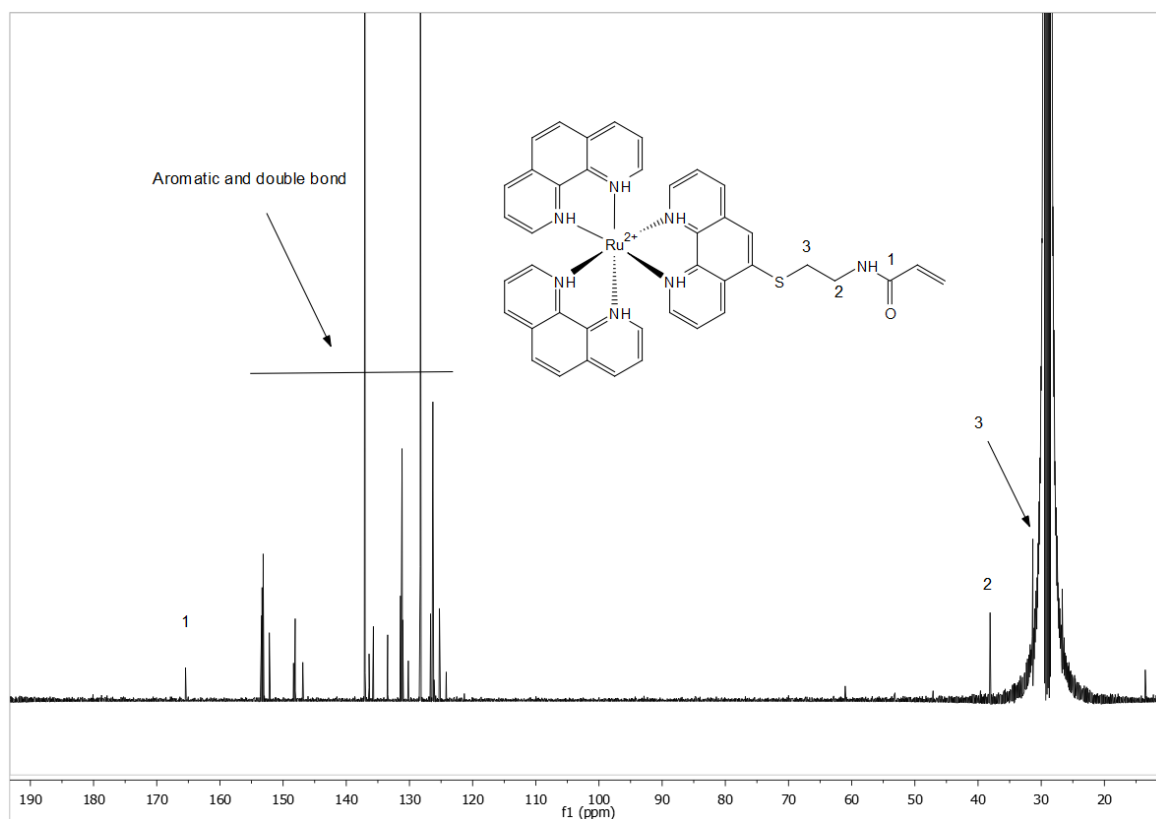
Fig. SI 10: ¹H NMR of 2-isopropyl-5-methylphenyl acrylate (Thymol acrylate) (20).

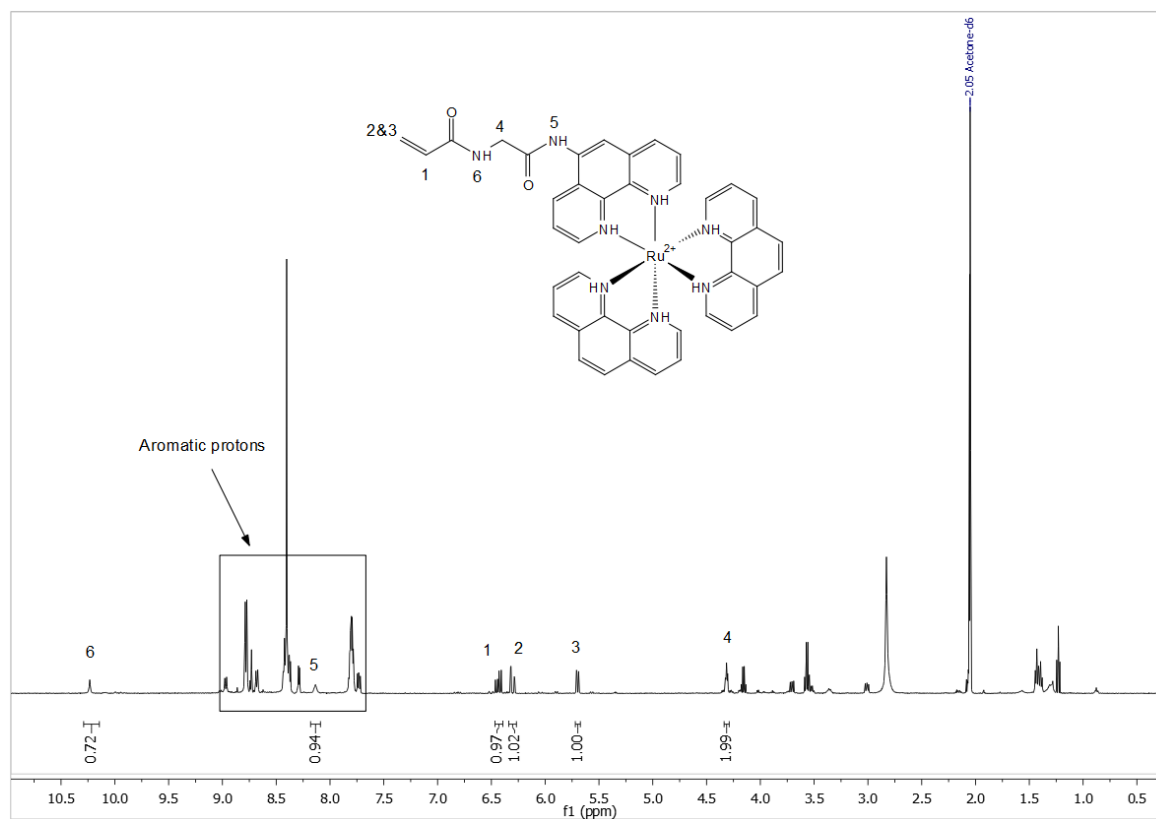
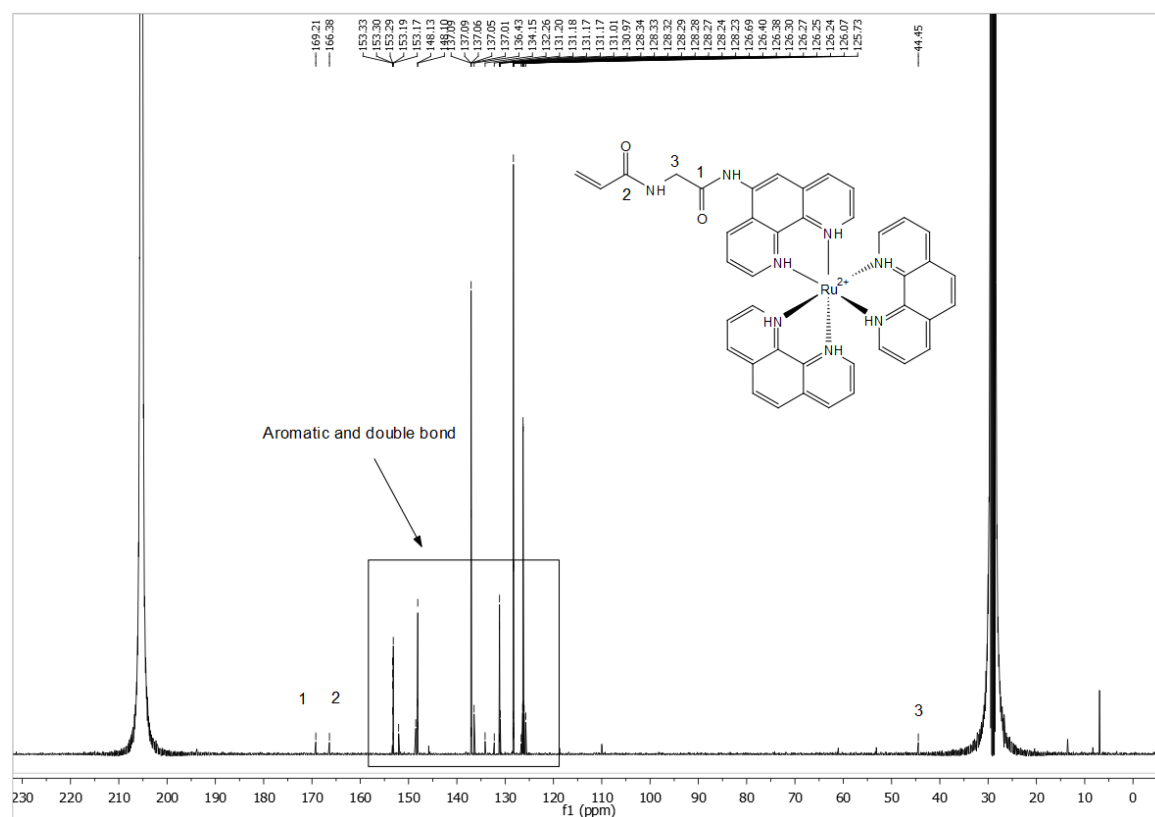
Fig. SI 11: ¹H NMR of N-(1,10-phenanthrolin-5-yl)ethane-1,2-diamine (**1**).Fig. SI 12: ¹H NMR of *tert*-butyl (2-((1,10-phenanthrolin-5-yl)amino)-2-oxoethyl)carbamate (**2**).

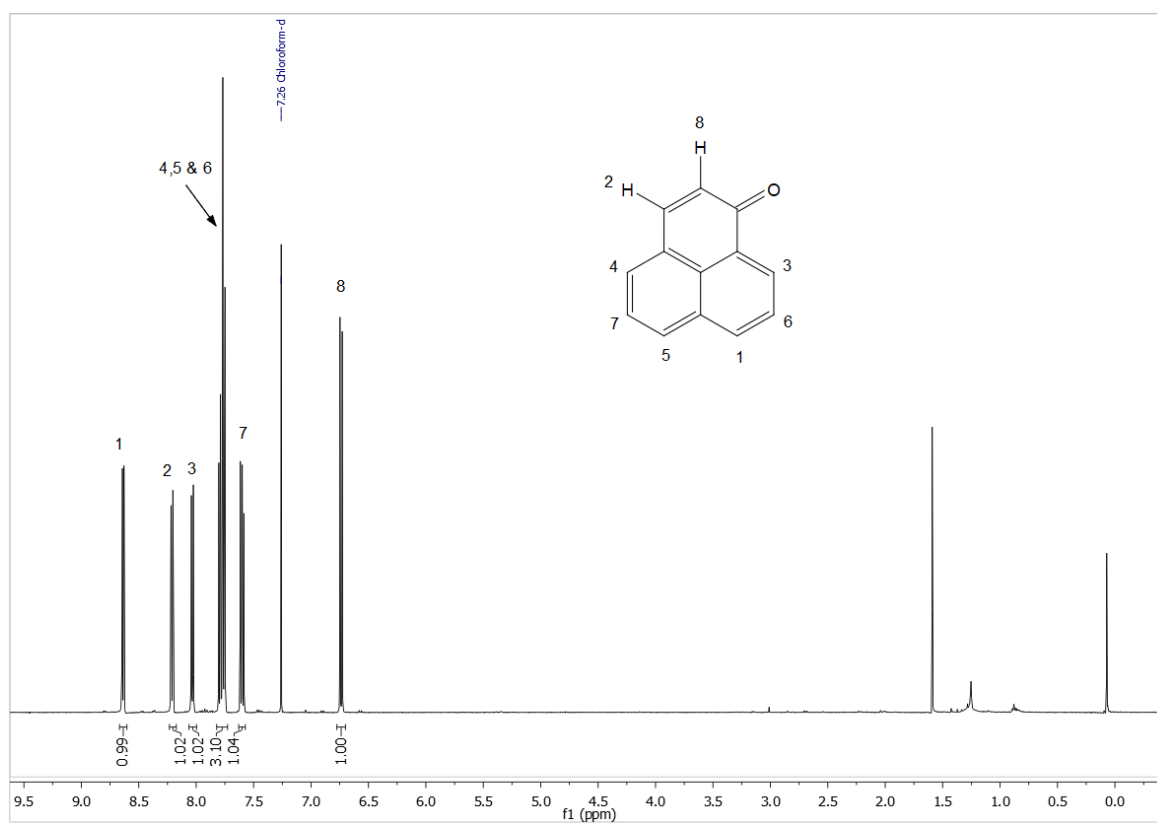
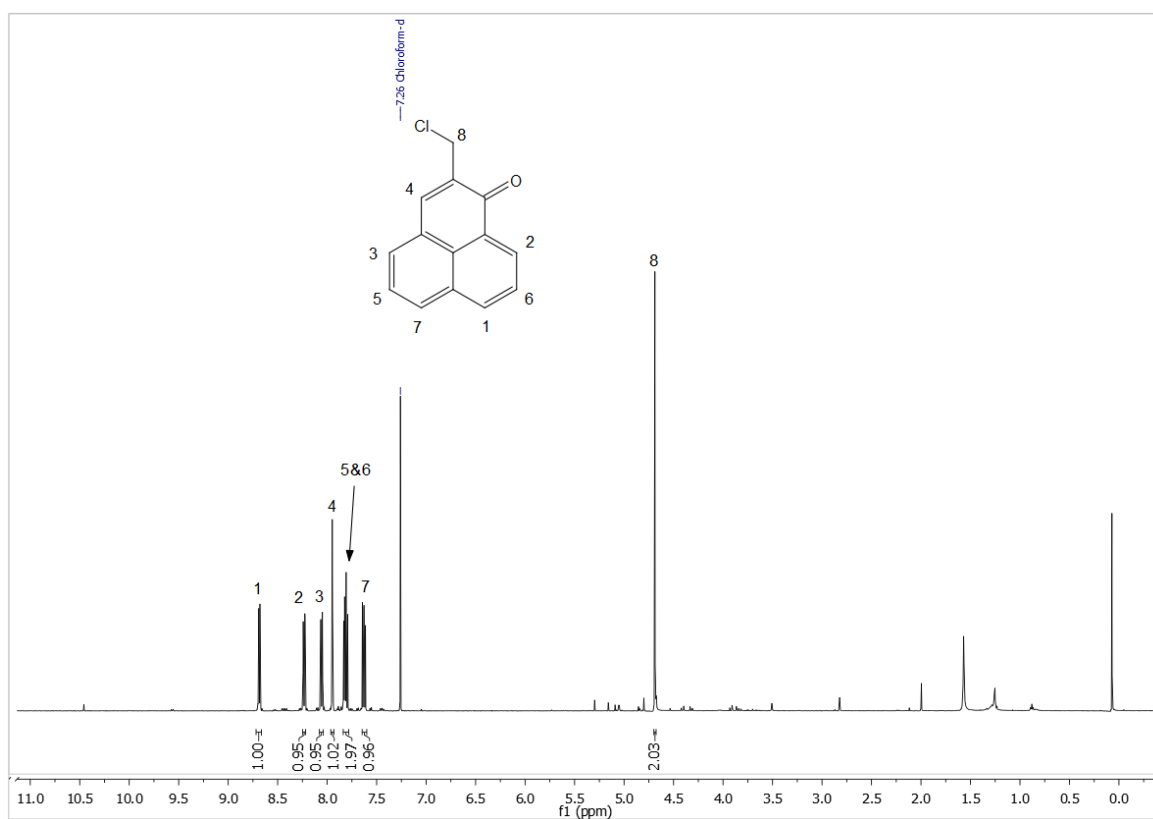
Fig. SI 13: ^1H NMR of 2-((1,10-phenanthroline-5-yl)thio)ethanamine (**3**).Fig. SI 14: ^1H NMR of Ru(1,10-Phenanthroline)₂Cl₂ (**5**).

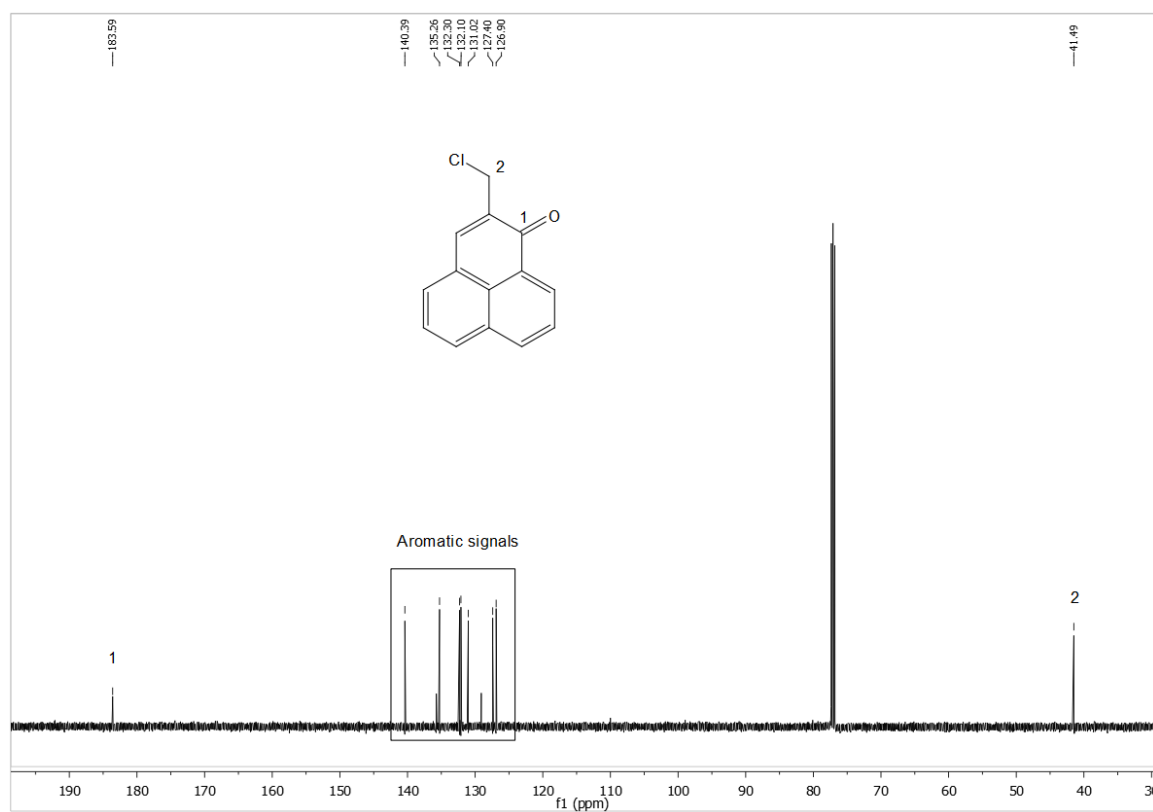
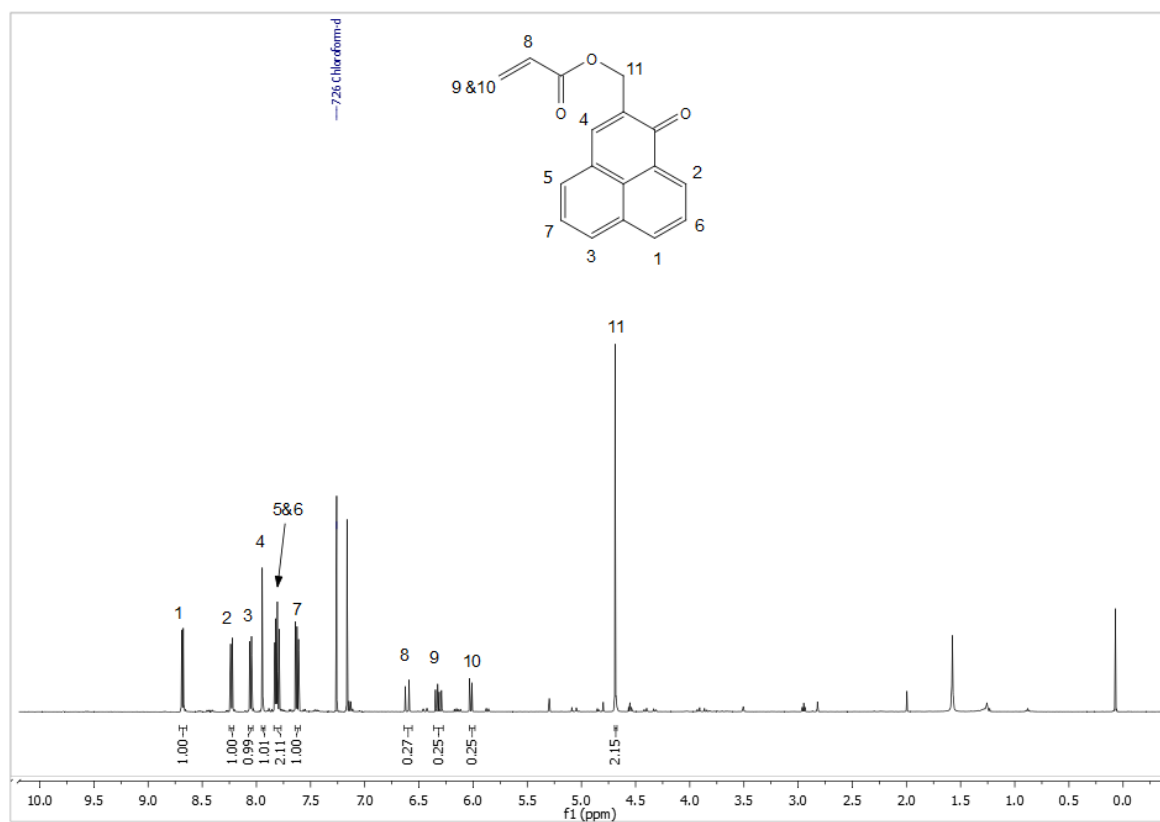
Fig. SI 15: ^1H NMR of $[\text{Ru}(\text{phen})_2(\text{phen-NH}_2)]\text{Cl}_2$.Fig. SI 16: ^1H NMR of $[\text{Ru}(\text{phen})_2(\text{phen-NH}_2)](\text{PF}_6)_2$.

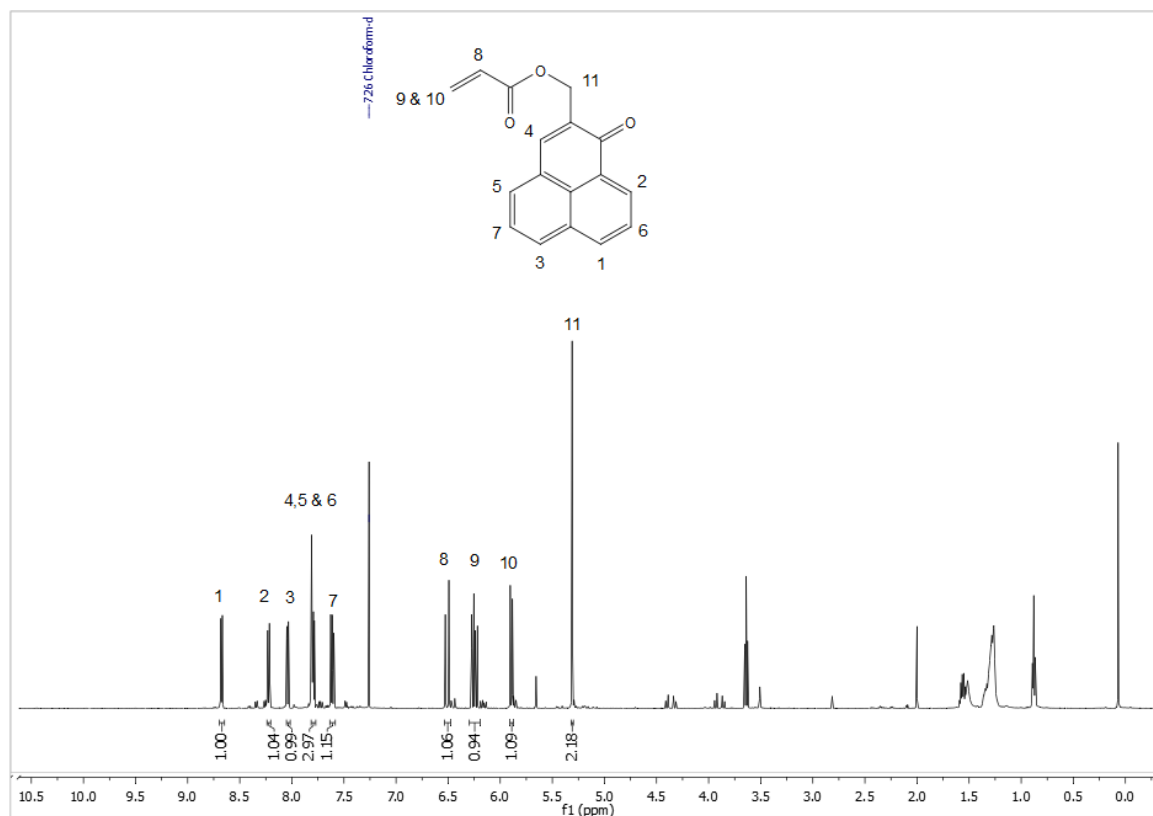
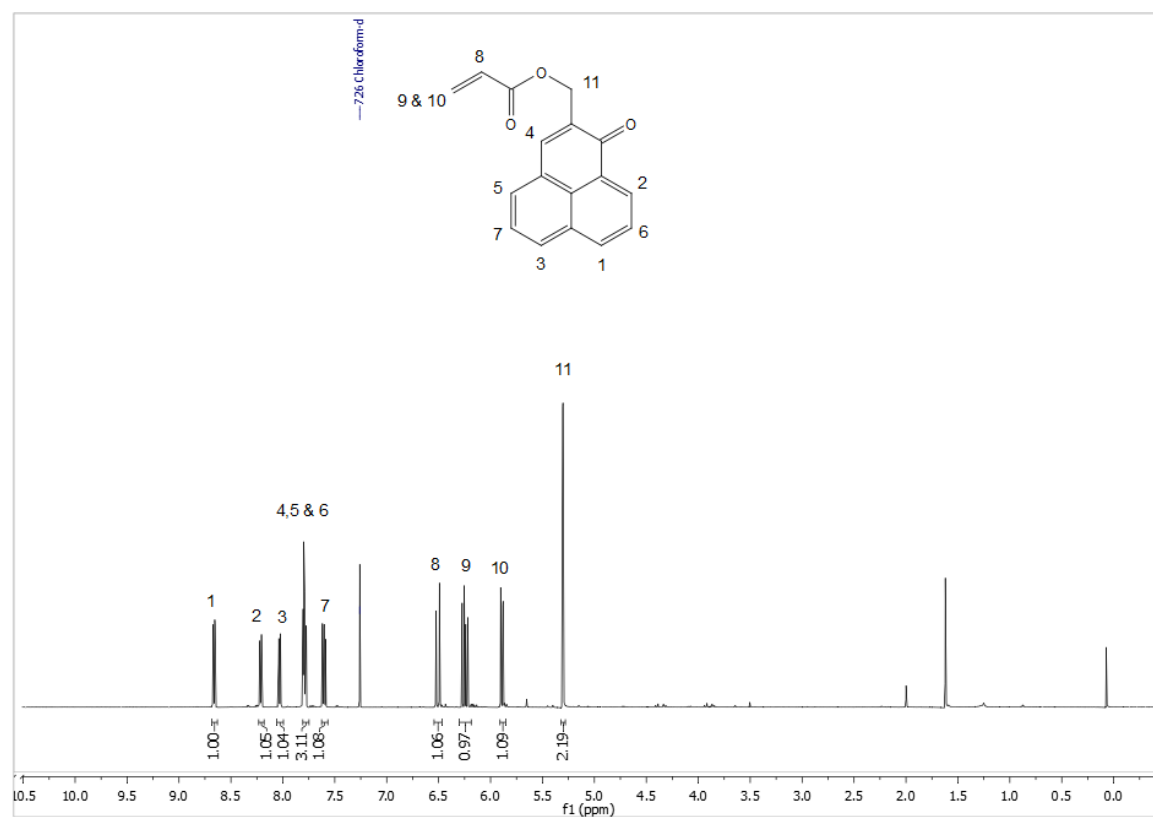
Fig. SI 17: ¹H NMR of [Ru(phen)₂(phen-amino-2-oxoethyl)carbamate] (PF₆)₂ (7).Fig. SI 18: ¹H NMR of [Ru(phen)₂(phen-thio-ethanamine)] (PF₆)₂ (8).

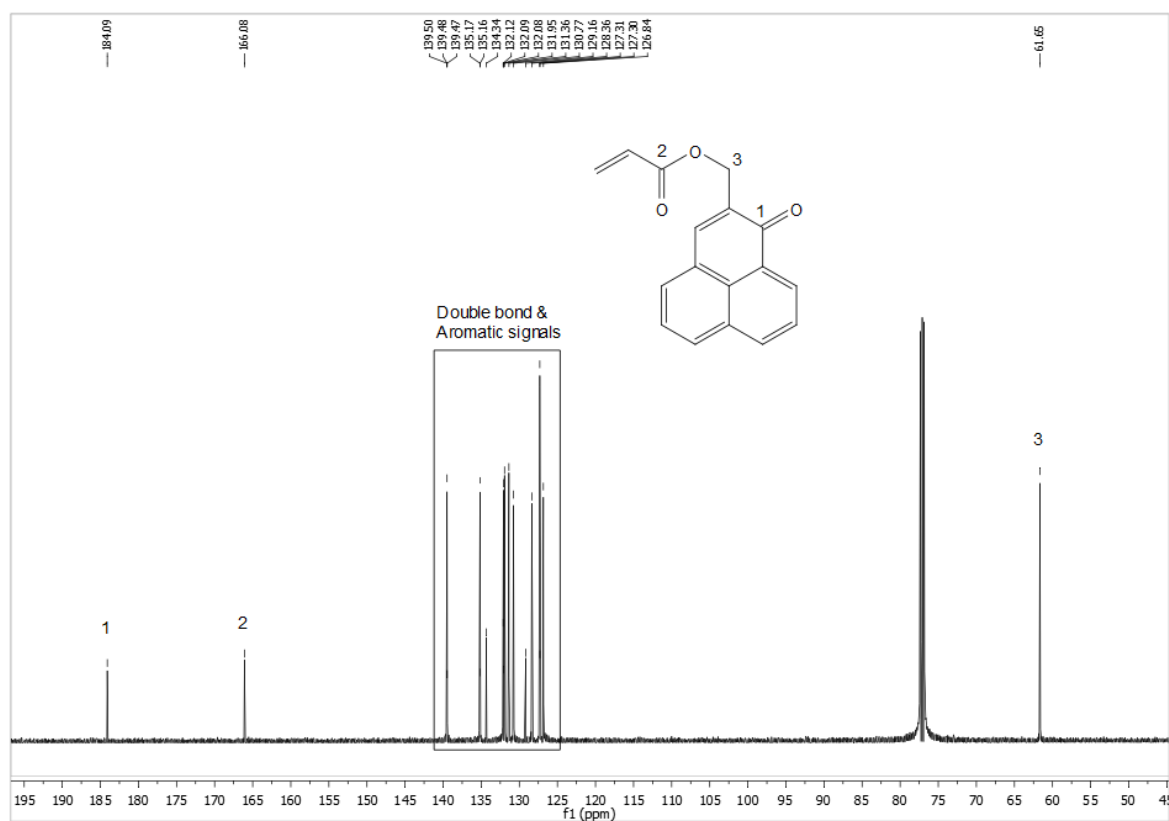
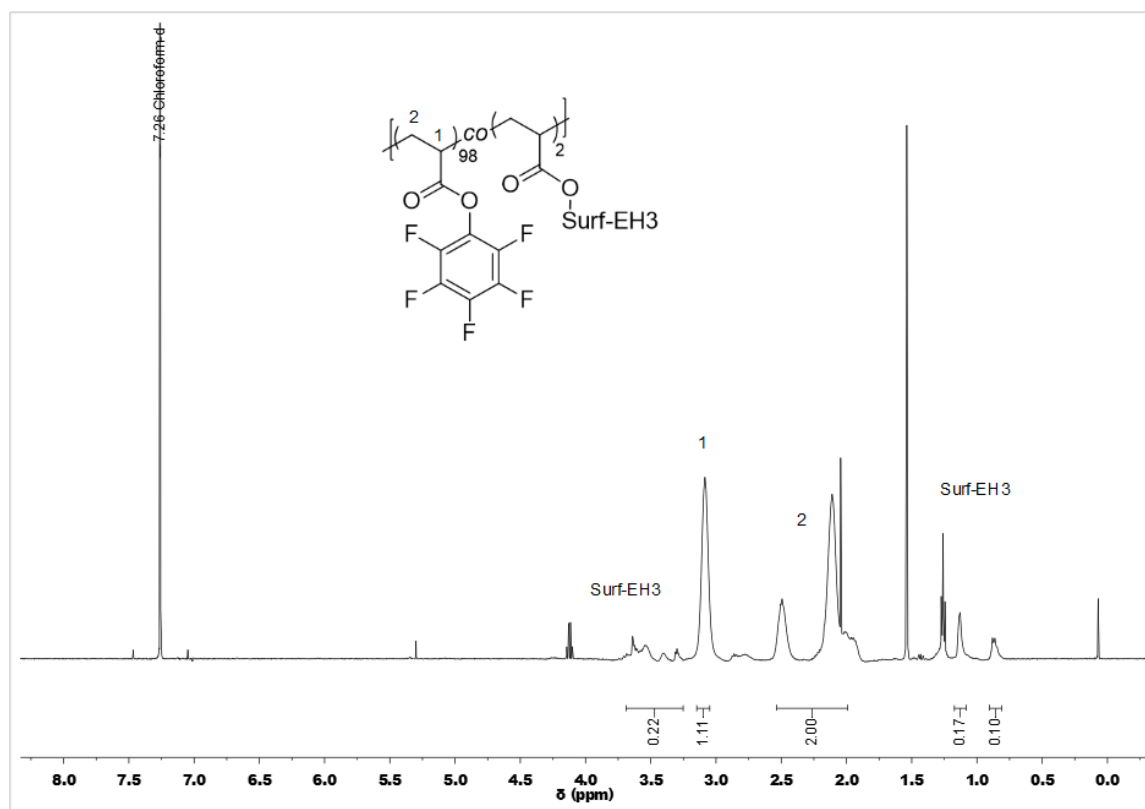
Fig. SI 19: ^1H NMR of Ru(phen) $_2$ (phen)acrylamide (**11**).Fig. SI 20: ^{13}C NMR of Ru(Phen) $_2$ (phen-thioethylacrylamide) (PF_6) $_2$ (**12**).

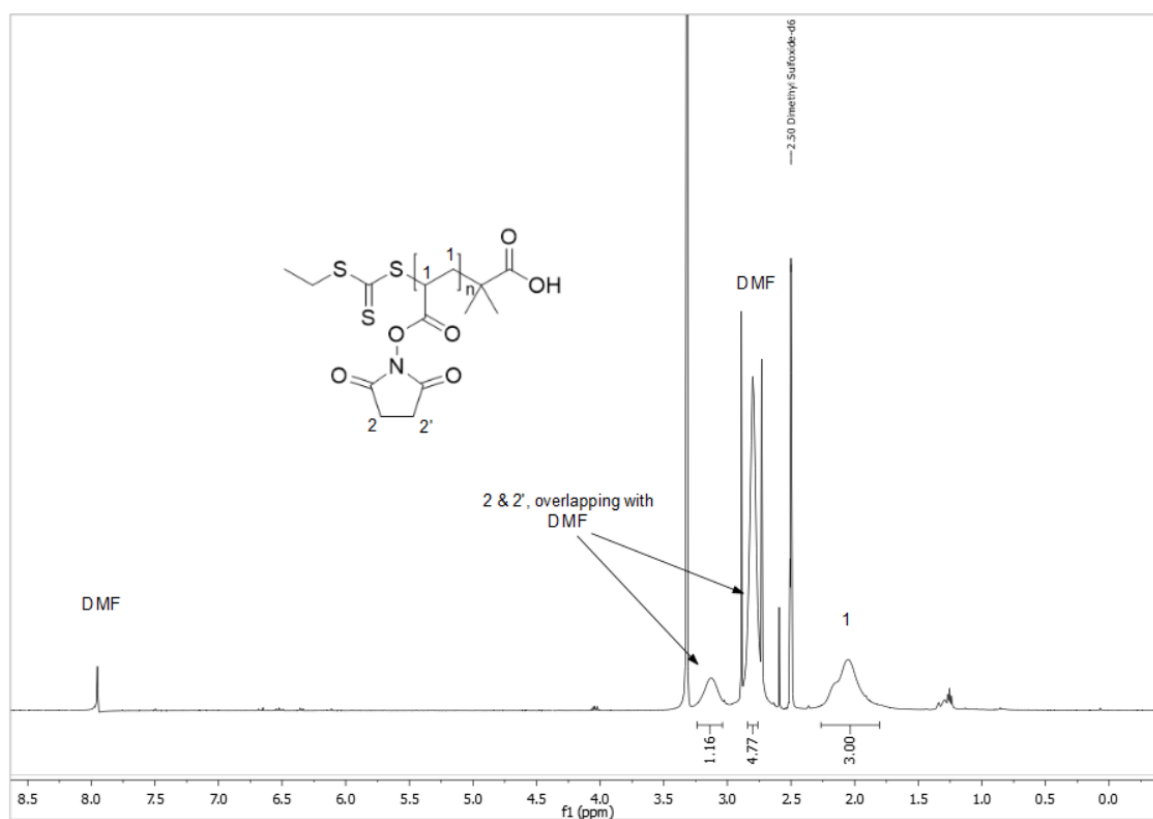
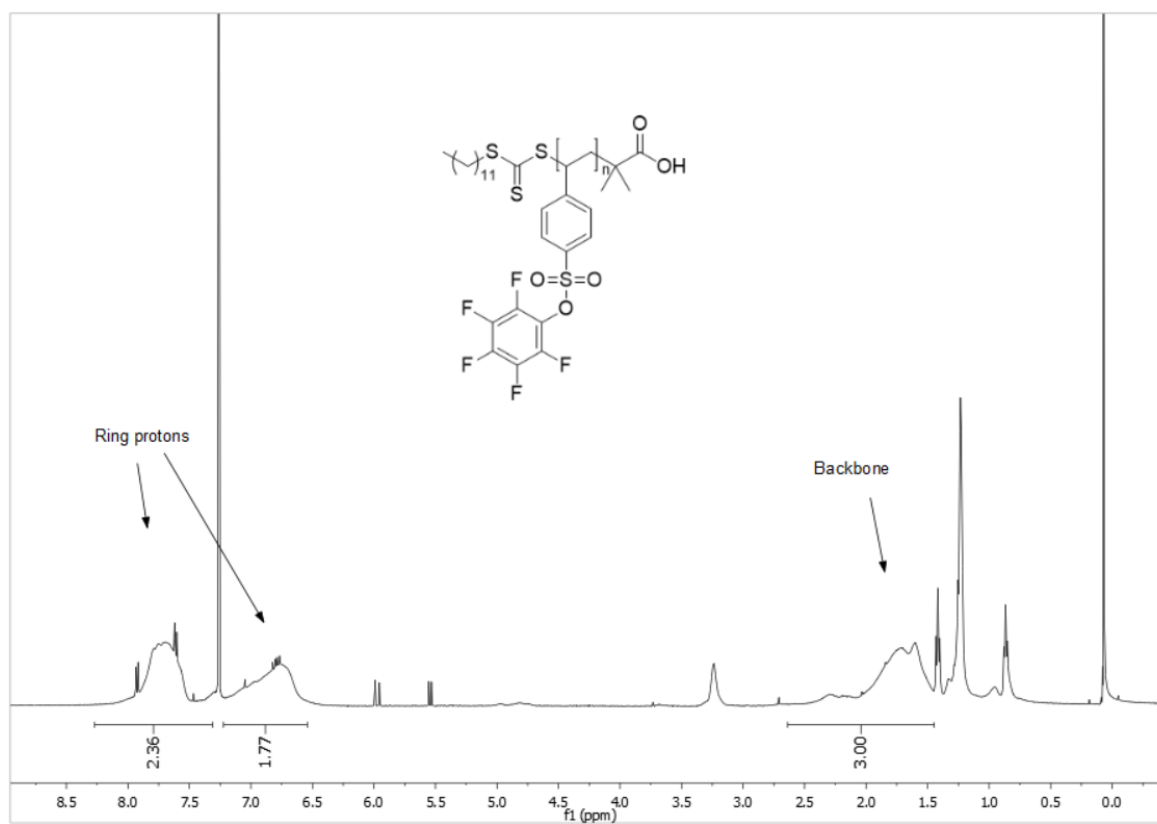
Fig. SI 21: ^1H NMR of $\text{Ru}(\text{Phen})_2(\text{phen-amino-2-oxoethylacrylamide}) (\text{PF}_6)_2$ (**13**).Fig. SI 22: ^{13}C NMR of $\text{Ru}(\text{Phen})_2(\text{phen-amino-2-oxoethylacrylamide}) (\text{PF}_6)_2$ (**13**).

Fig. SI 23: ¹H NMR of phenalene-1-one (**14**).Fig. SI 24: ¹H NMR of 2-(chloromethyl)-1H-phenalene-1-one (**15**).

Fig. SI 25: ^{13}C NMR of 2-(chloromethyl)-1H-phenalen-1-one (**15**).Fig. SI 26: ^1H NMR of (1-oxo-1H-phenalen-2-yl)methyl acrylate (**16**) variant A.

Fig. SI 27: ^1H NMR of (1-oxo-1H-phenalen-2-yl)methyl acrylate (**16**) variant B.Fig. SI 28: ^1H NMR of (1-oxo-1H-phenalen-2-yl)methyl acrylate (**16**) variant C.

Fig. SI 29: ^{13}C NMR of (1-oxo-1H-phenalen-2-yl)methyl acrylate (**16**) variant C.Fig. SI 30: ^1H NMR of polypentafluorophenol-co-Surf-EH3 (**25**).

Fig. SI 31: ^1H NMR of polyN-hydroxysuccinimide acrylate.Fig. SI 32: ^1H NMR of polypentafluorophenol-4-vinylbenzene sulfonate.

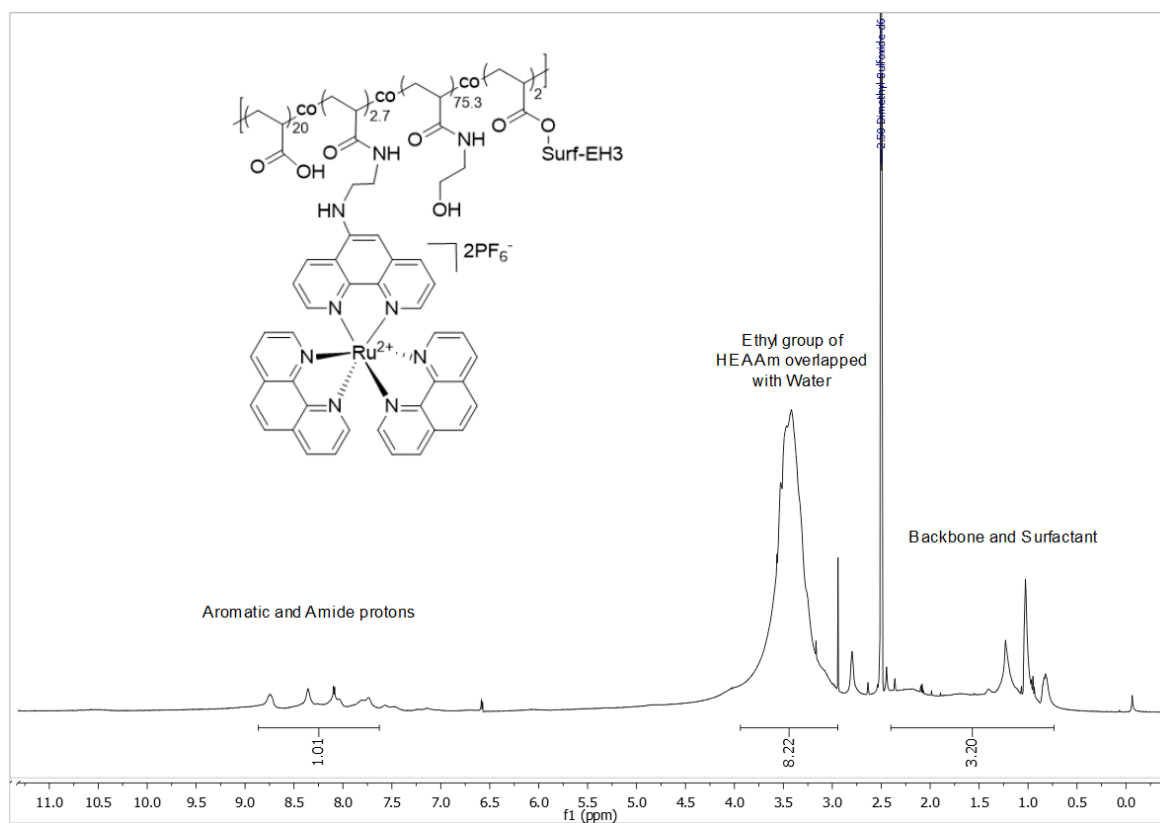


Fig. SI 33: ^1H NMR of poly(AA₂₀-co-Ru(NH₂)_{2.7}-co-HEAAm_{75.3}-co-SurfEH3A₂) (**26**).

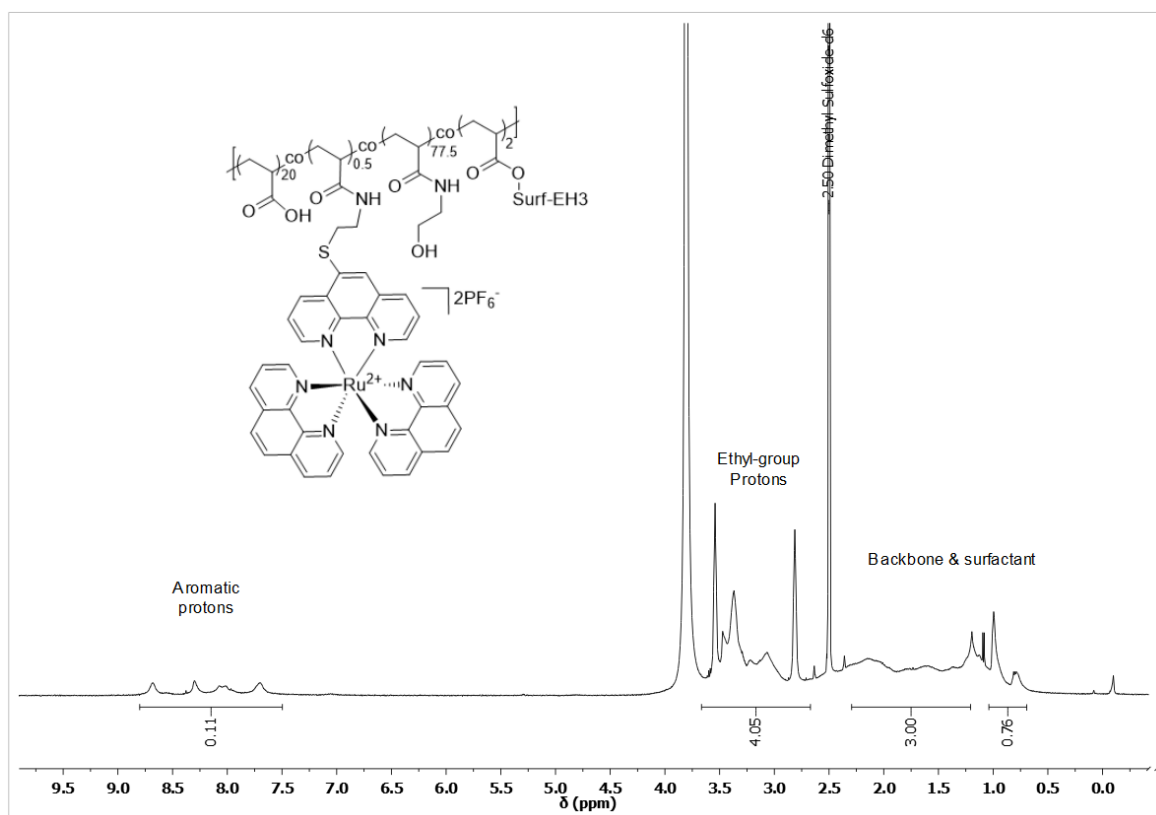
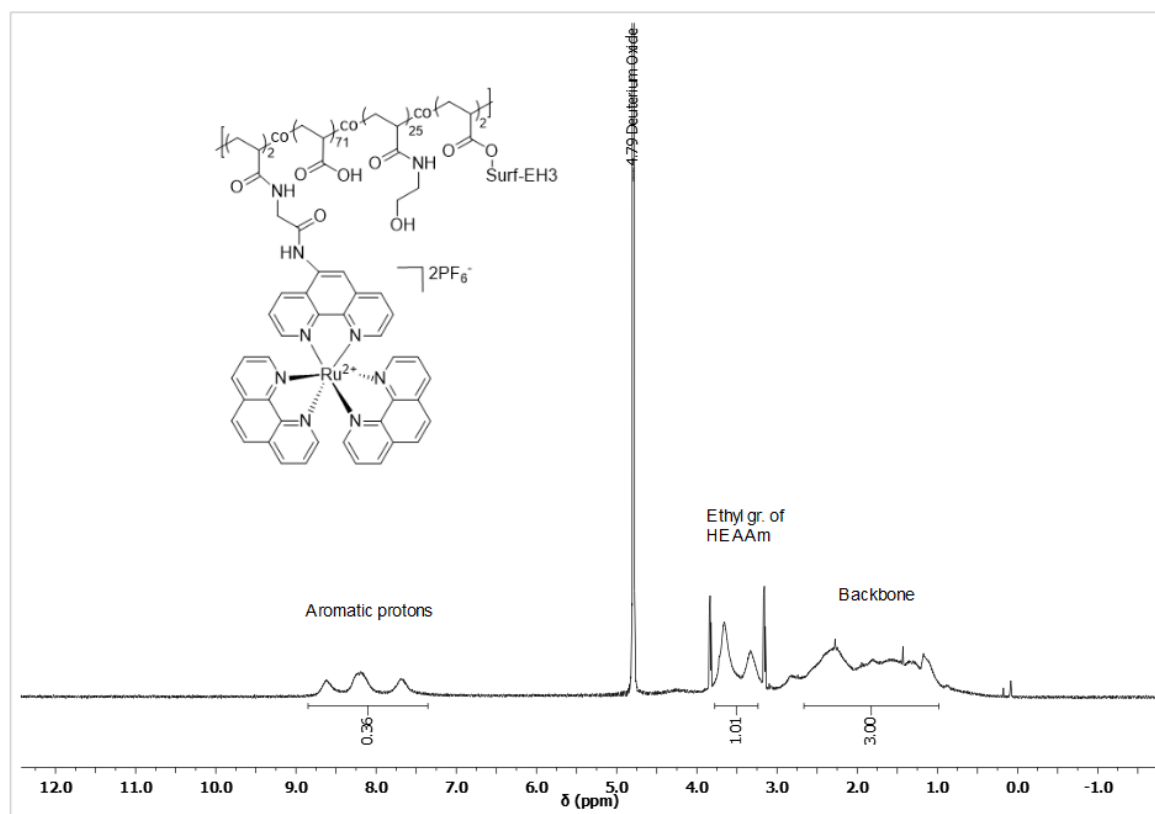
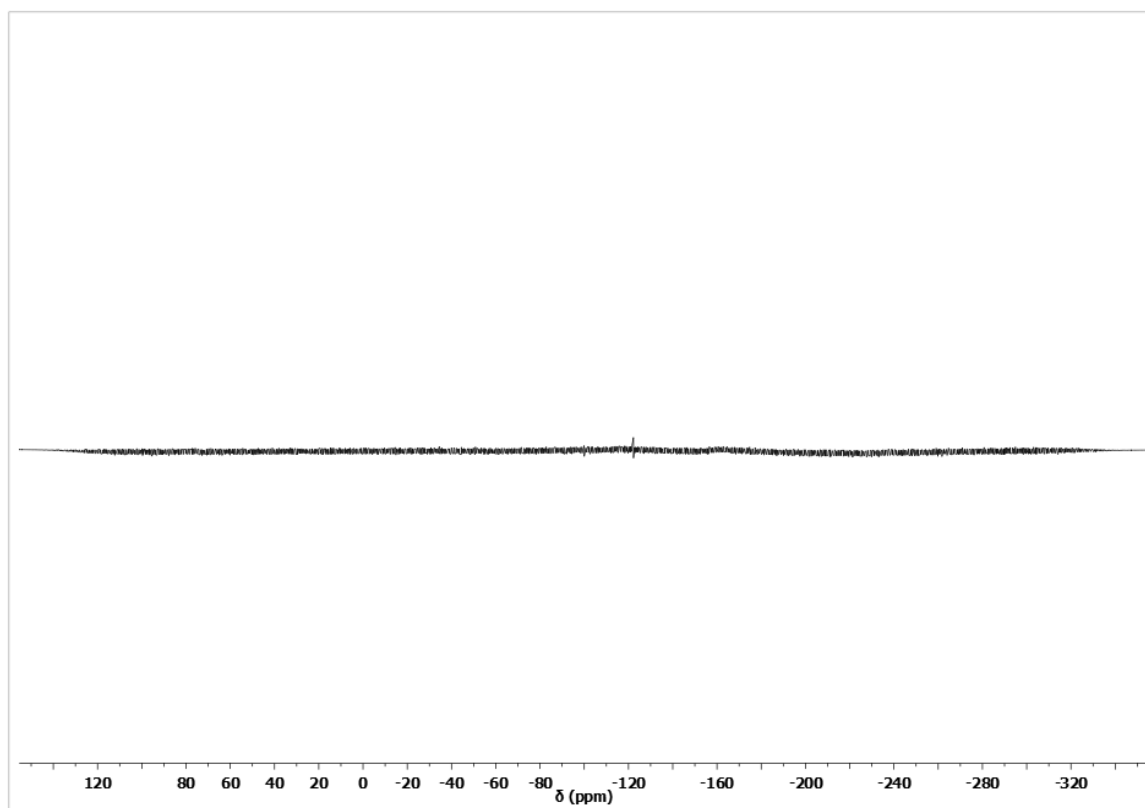
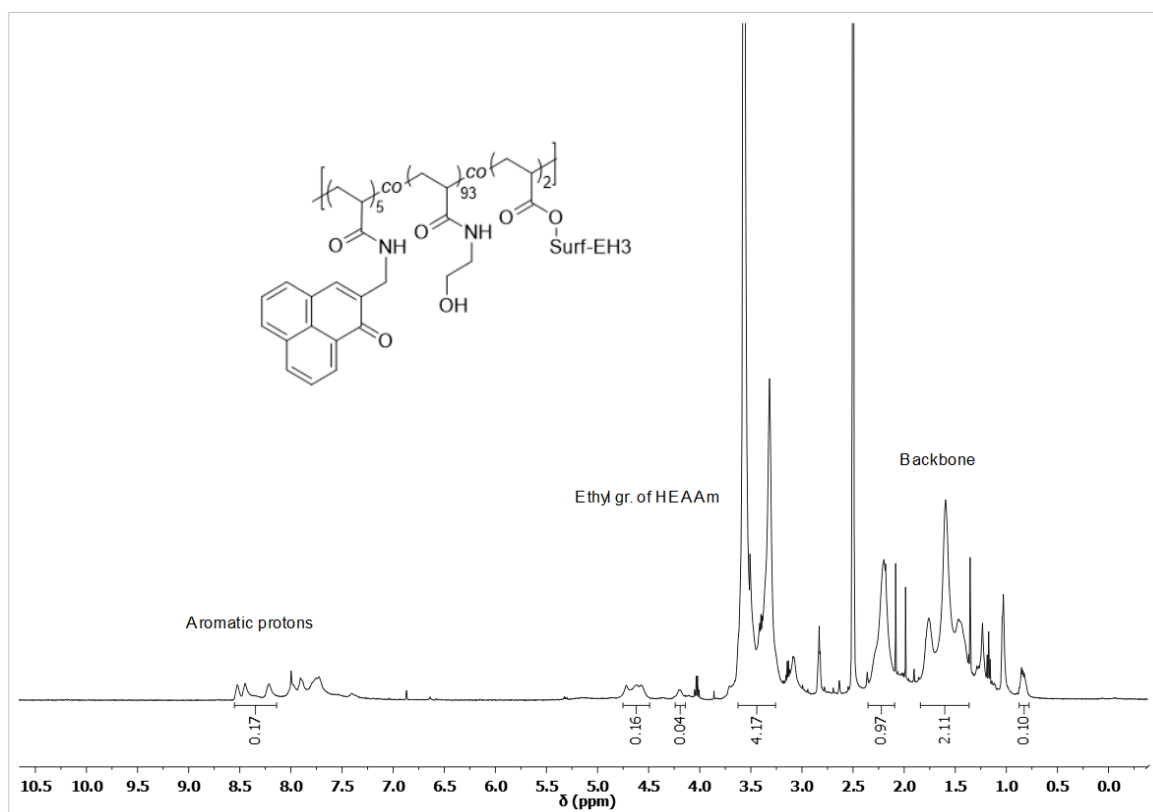
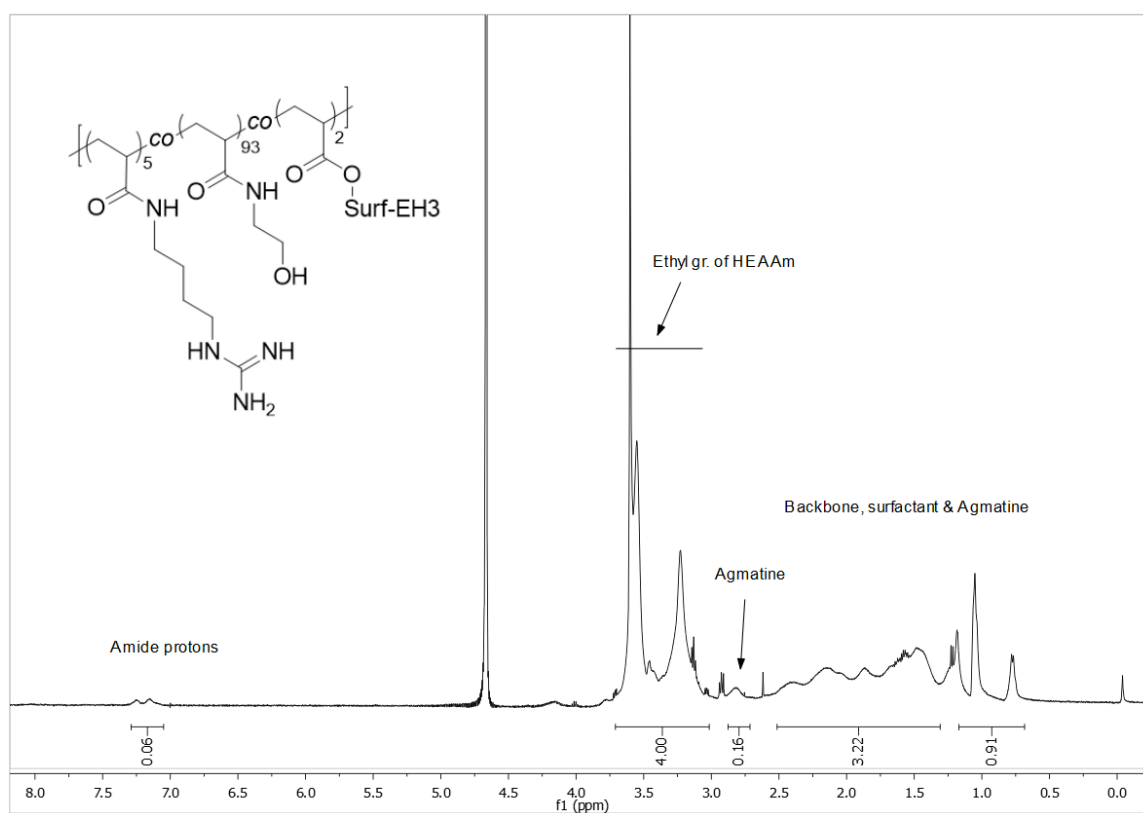
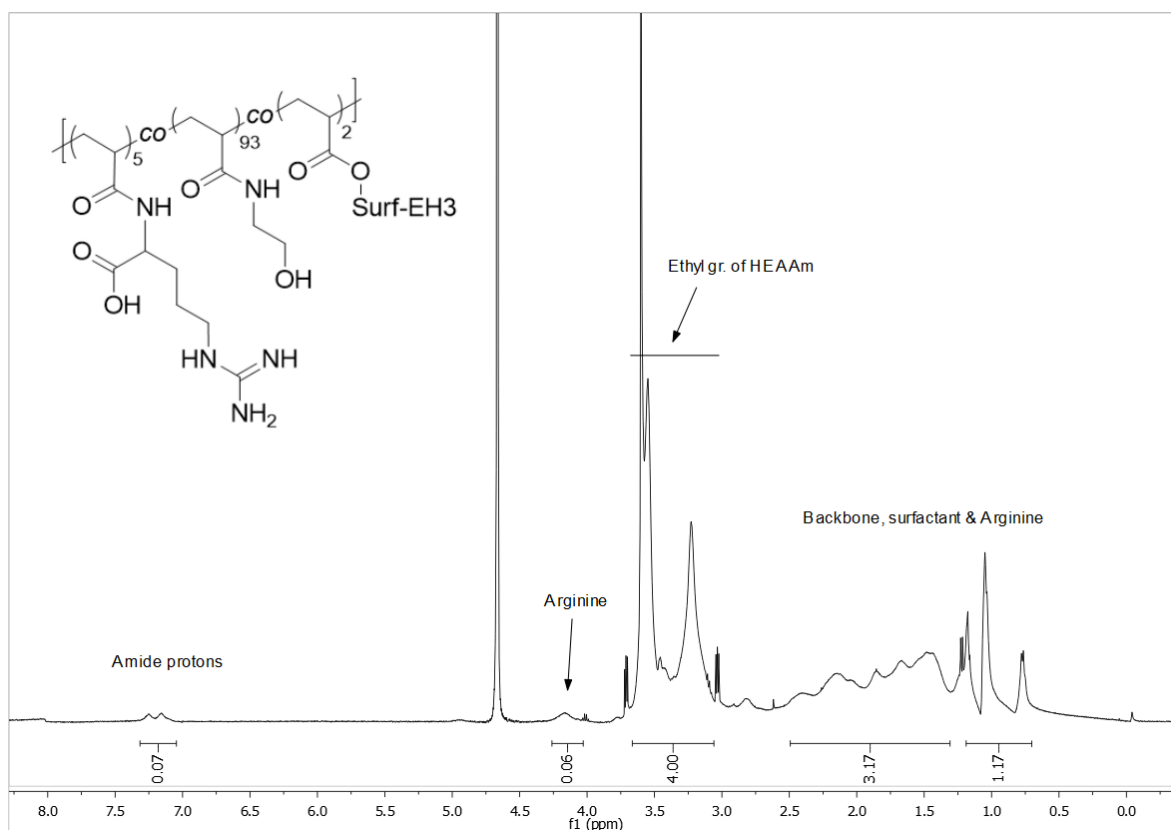
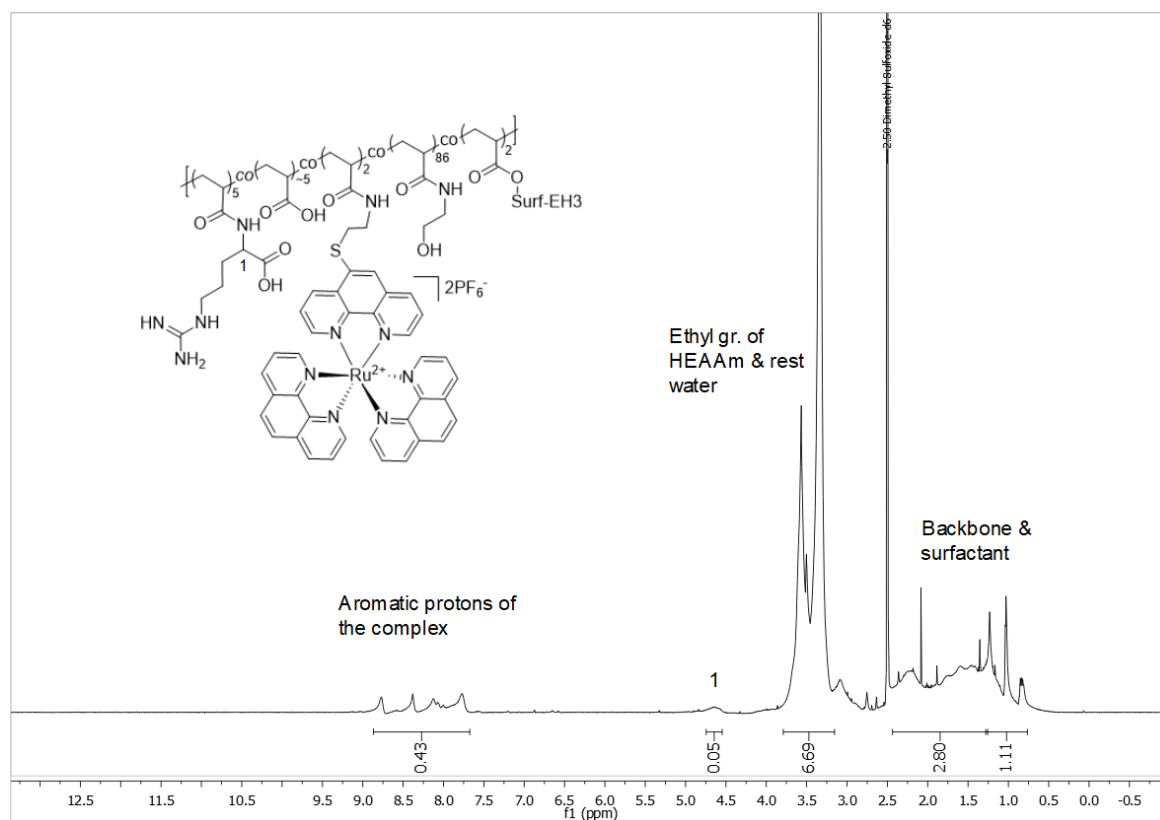
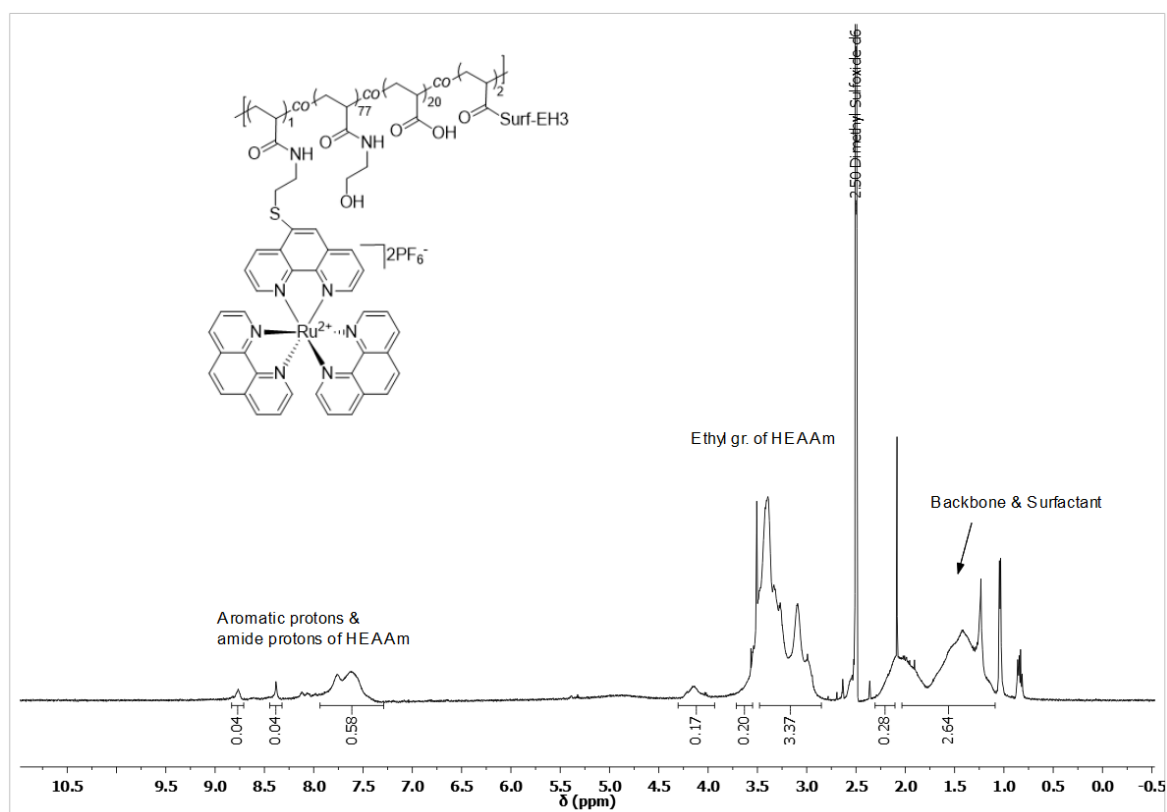
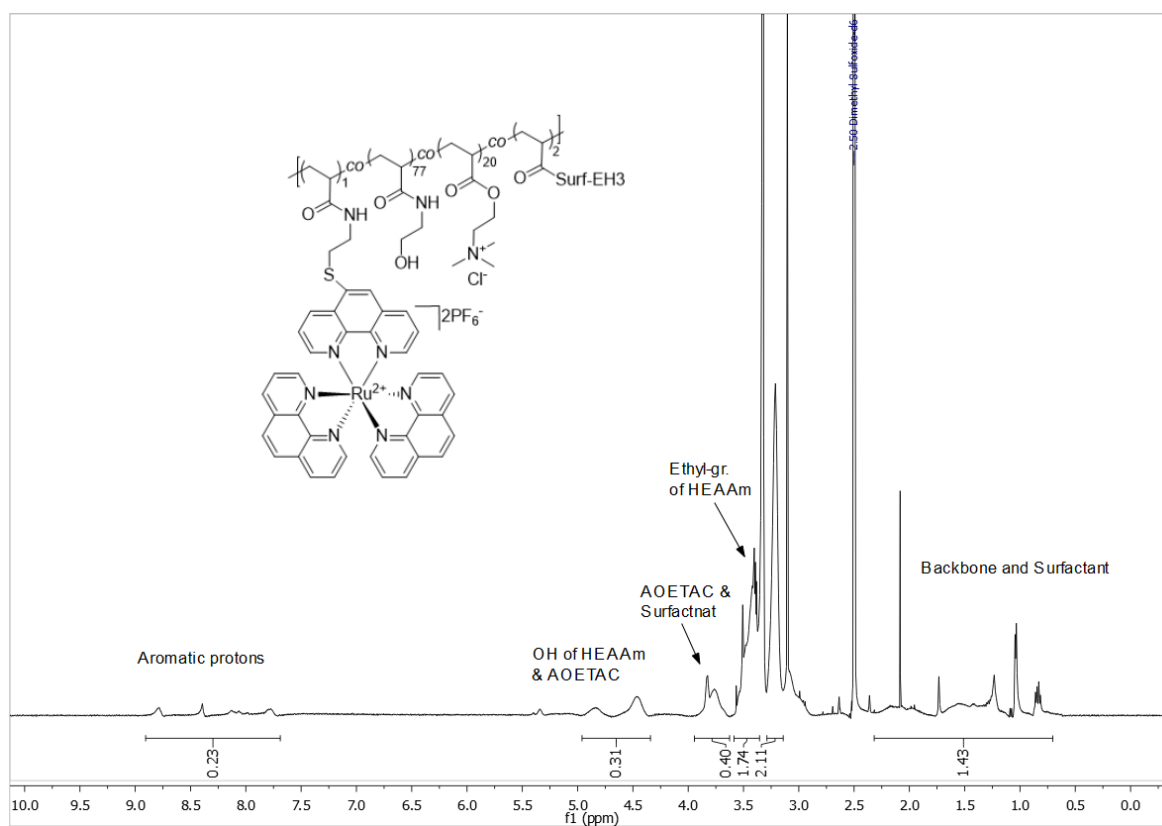


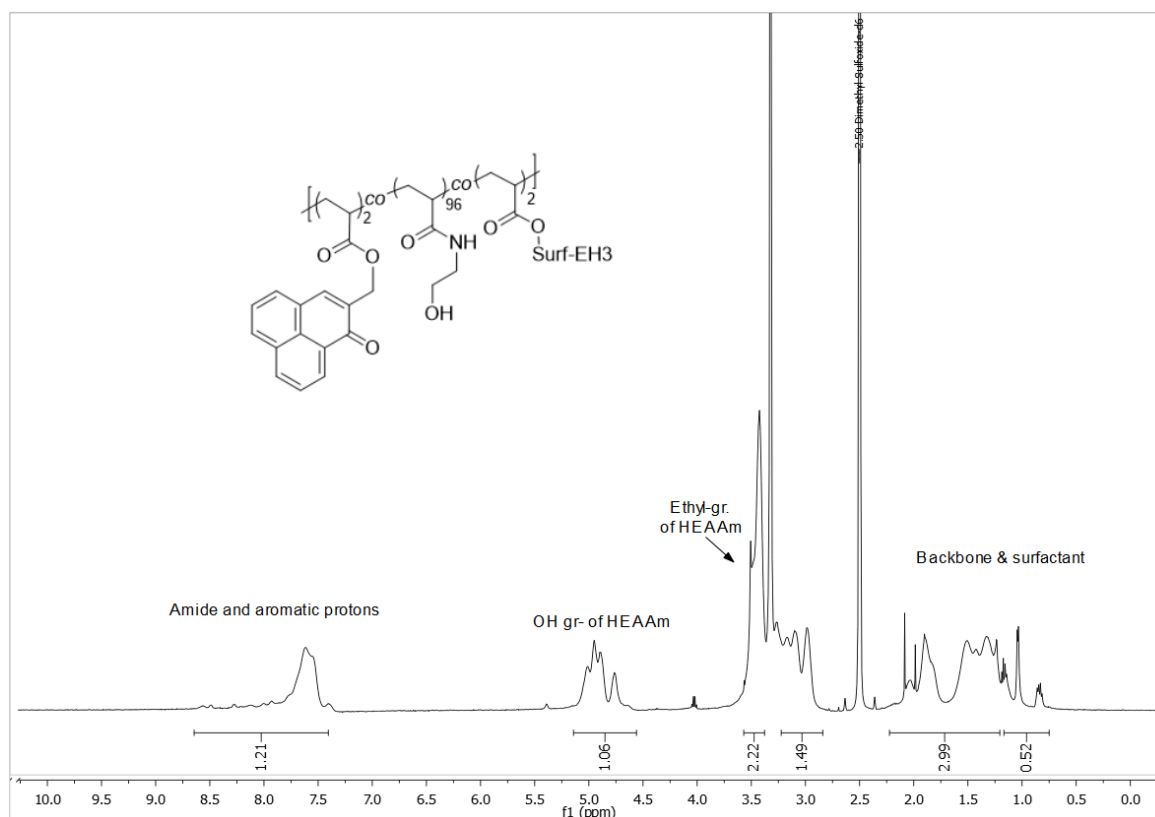
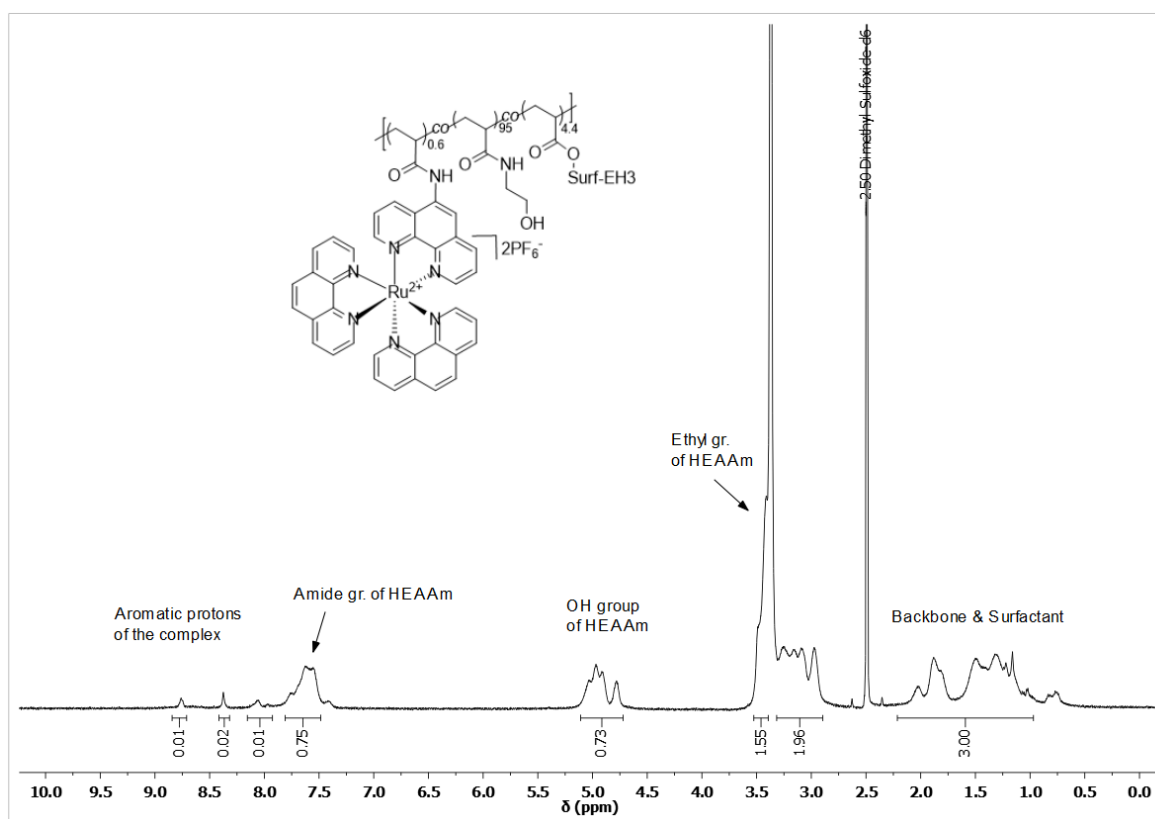
Fig. SI 34: ^1H NMR of poly(AA₂₀-co-Ru(S)_{0.5}-co-HEAAm_{77.5}-co-SurfEH3A₂) (**27**).

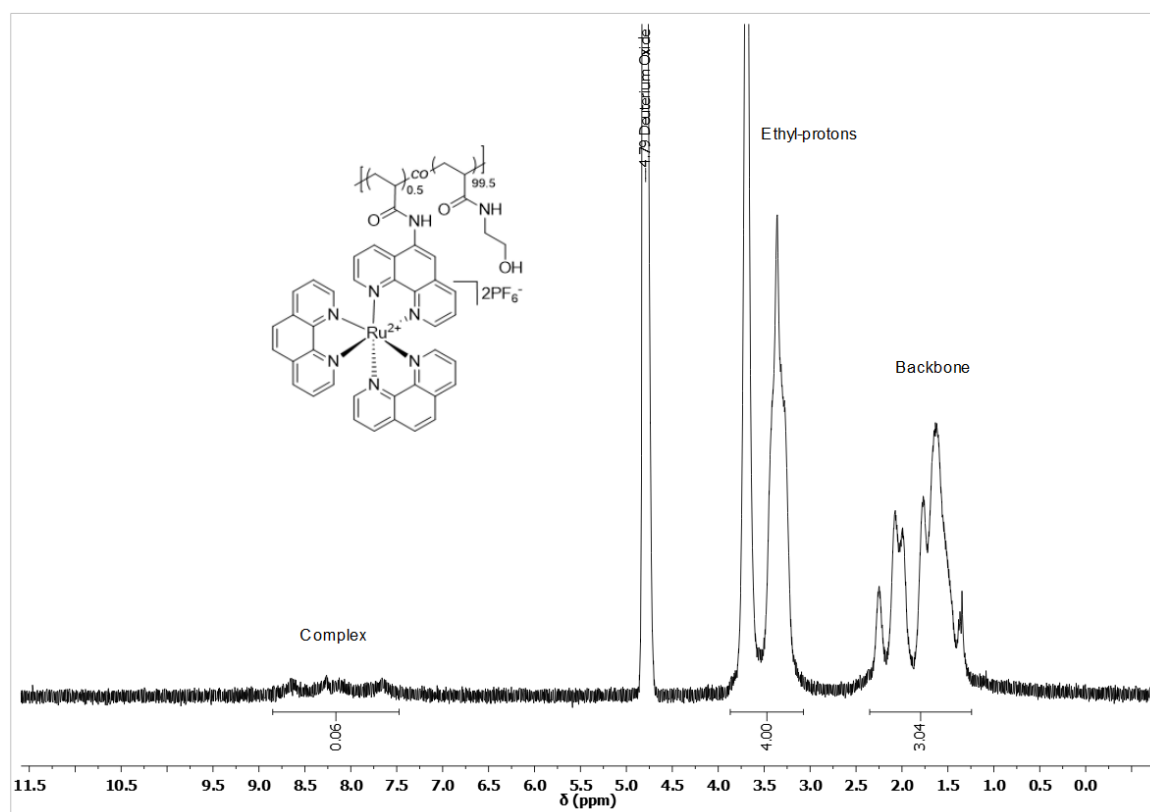
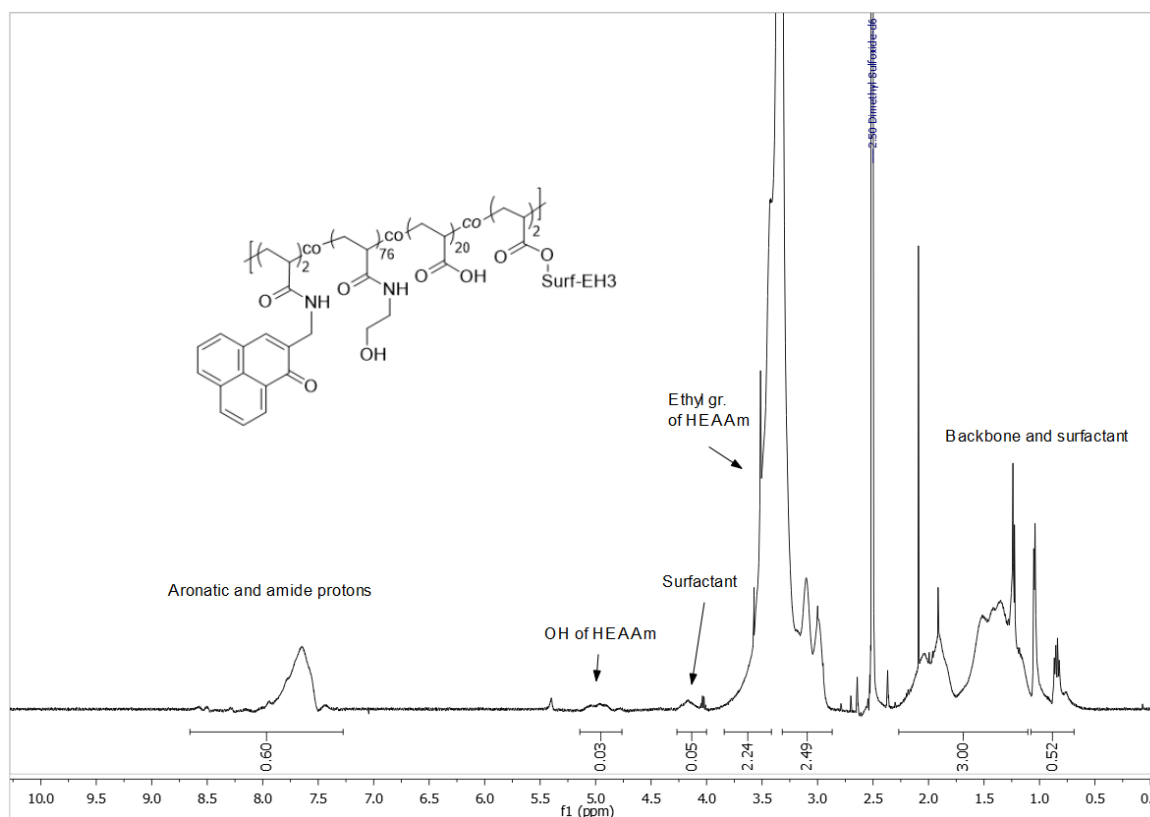
Fig. SI 35: ^1H NMR of $\text{poly}(\text{Ru}(\text{Boc})_2\text{-co-AA}_{71}\text{-co-HEAAm}_{25}\text{-co-Surf-EH3A}_2)$ (**28**).Fig. SI 36: ^{19}F NMR of $\text{poly}(\text{Ru}(\text{Boc})_2\text{-co-AA}_{71}\text{-co-HEAAm}_{25}\text{-co-Surf-EH3A}_2)$ (**28**).

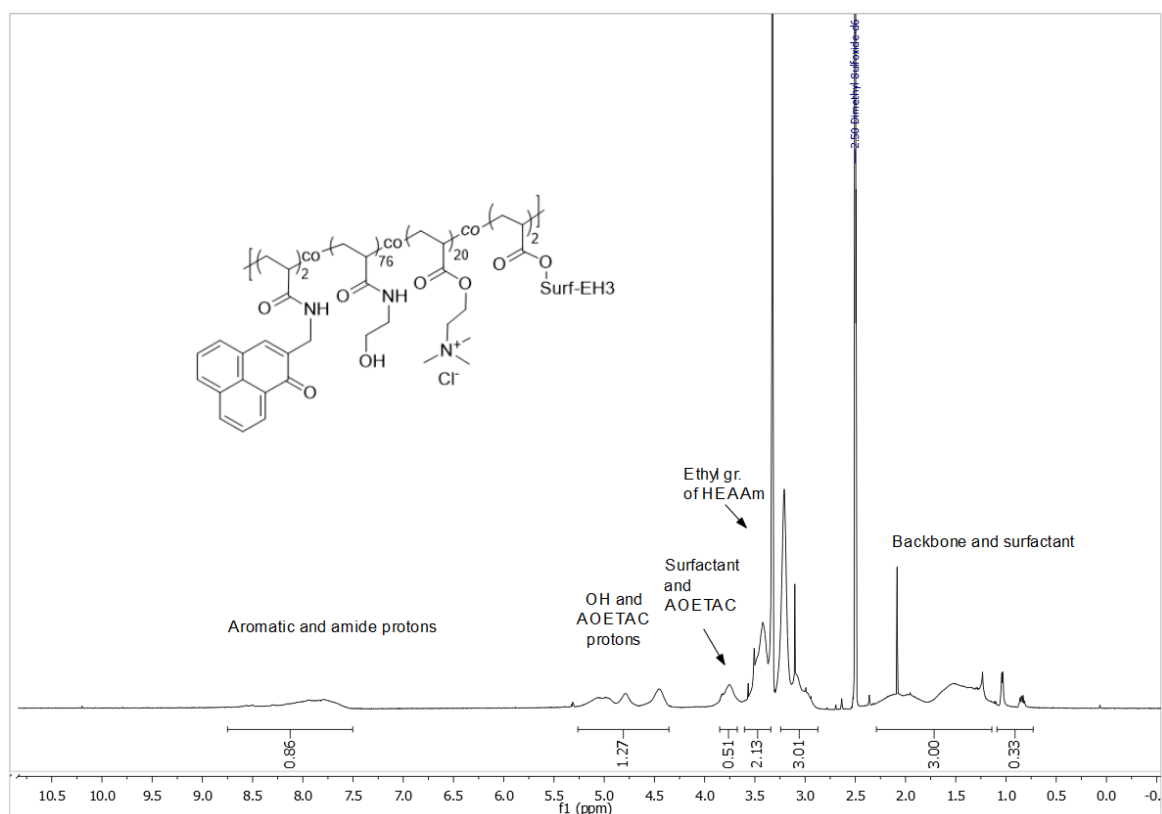
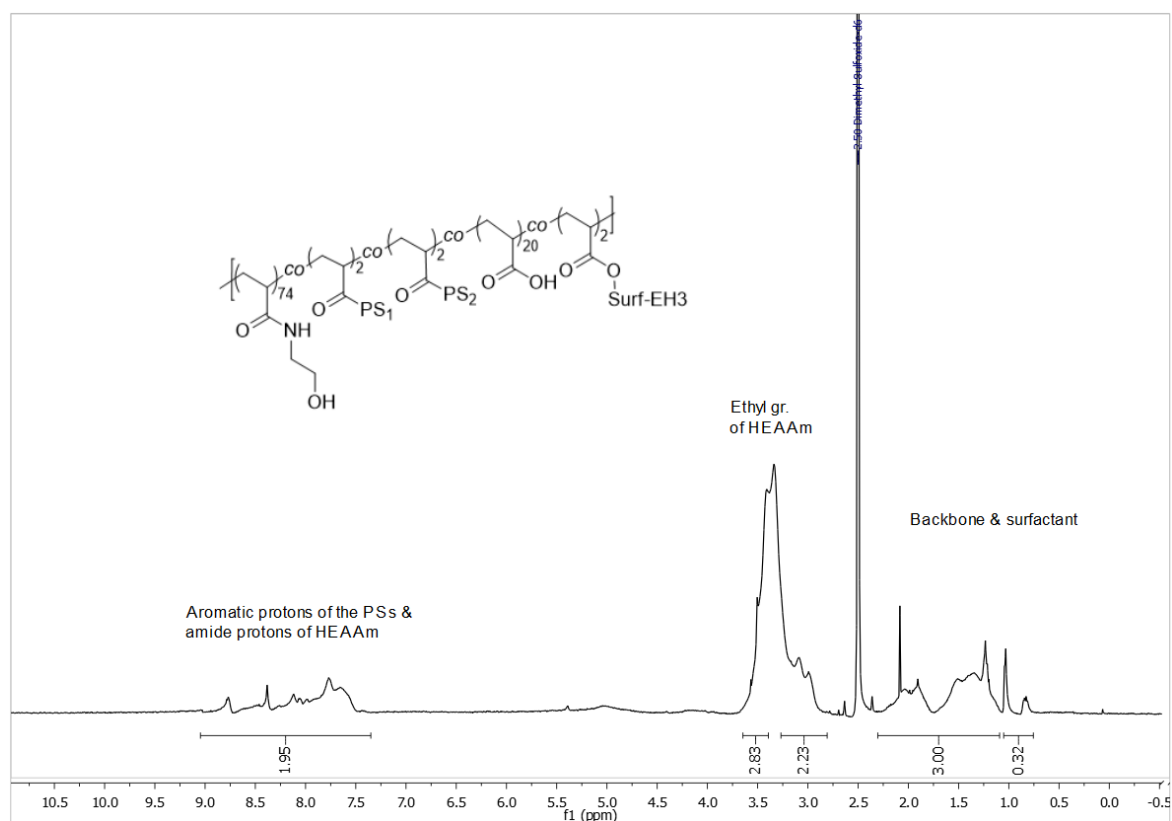
Fig. SI 37: ¹H NMR of poly(PN₅-co-HEAAm₉₃-co-Surf-EH3A₂) (29).Fig. SI 38: ¹H NMR of poly(Agm₅-co-HEAAm₉₃-co-Surf-EH3A₂) (30).

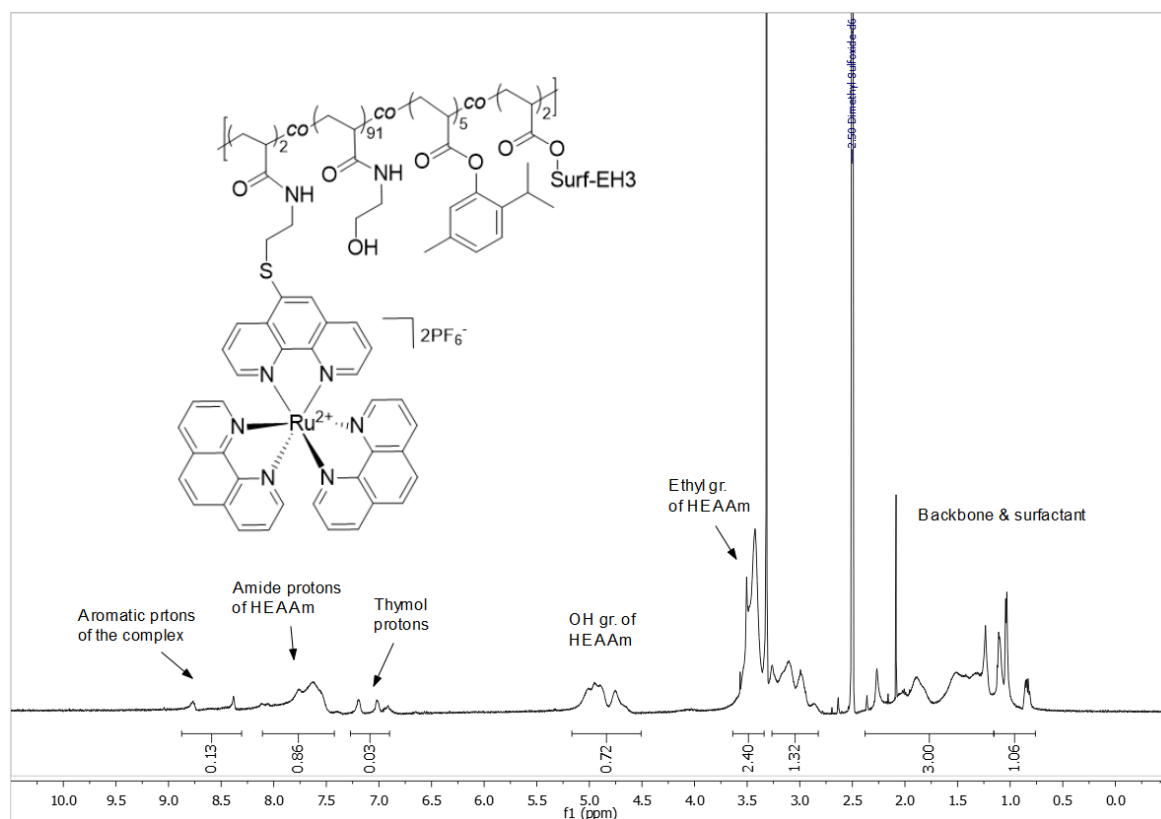
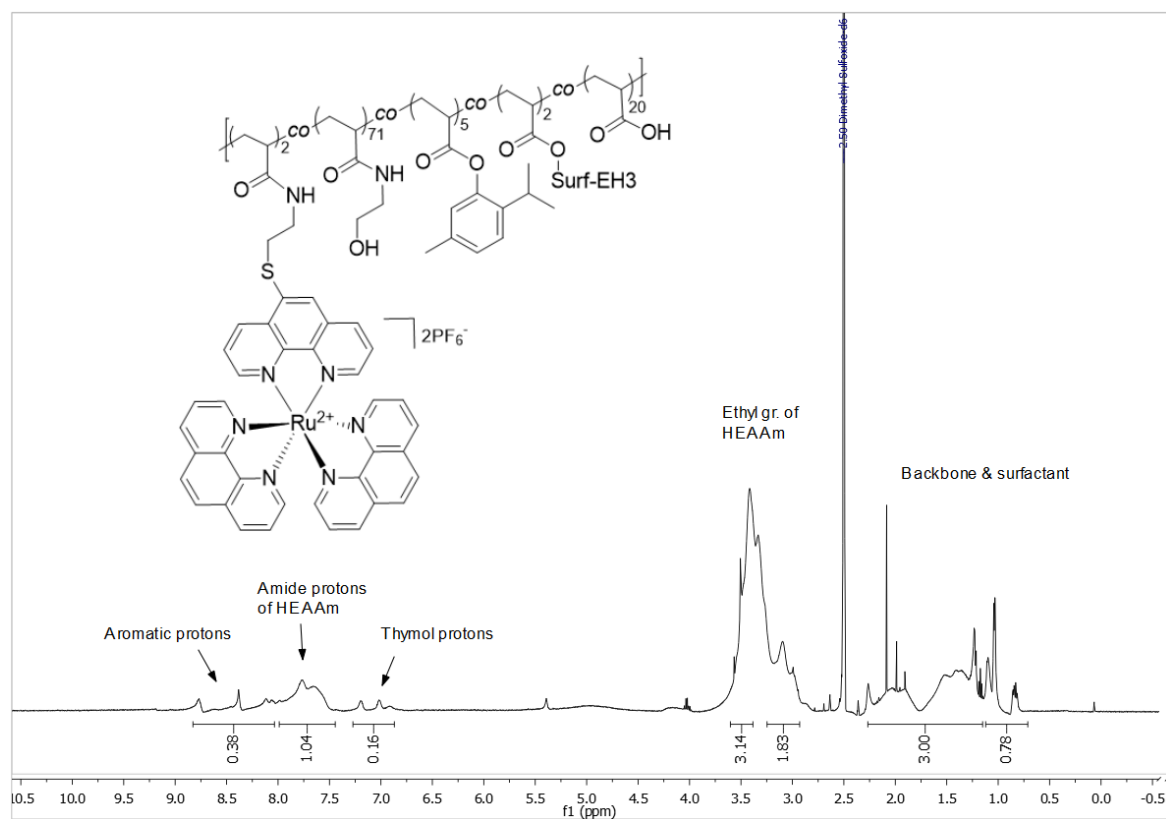
Fig. SI 39: ^1H NMR of $\text{poly}(\text{Arg}_5\text{-co-HEAAm}_{93}\text{-co-Surf-EH3A}_2)$ (31).Fig. SI 40: ^1H NMR of $\text{polyL-arginin}_5\text{-co-AA}_5\text{-co-Ru}(\text{S})_2\text{-co-HEAAm}_{86}\text{-co-Surf-EH3A}_2$ (32)

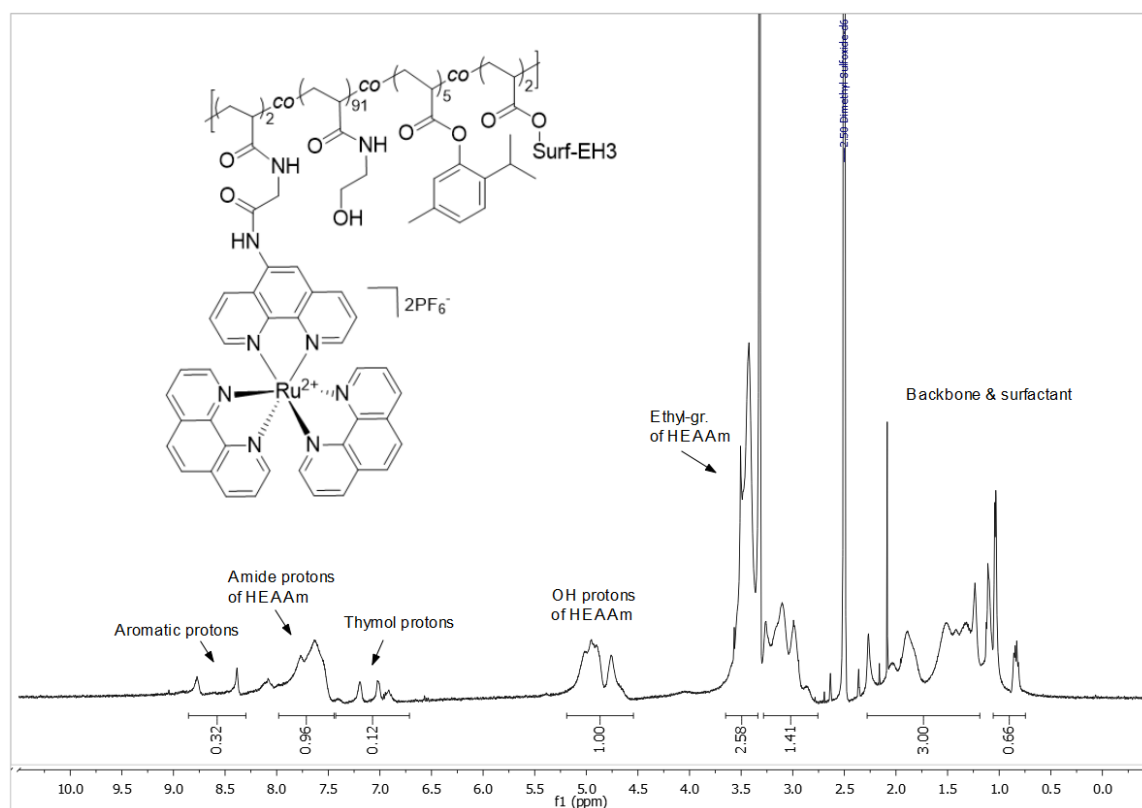
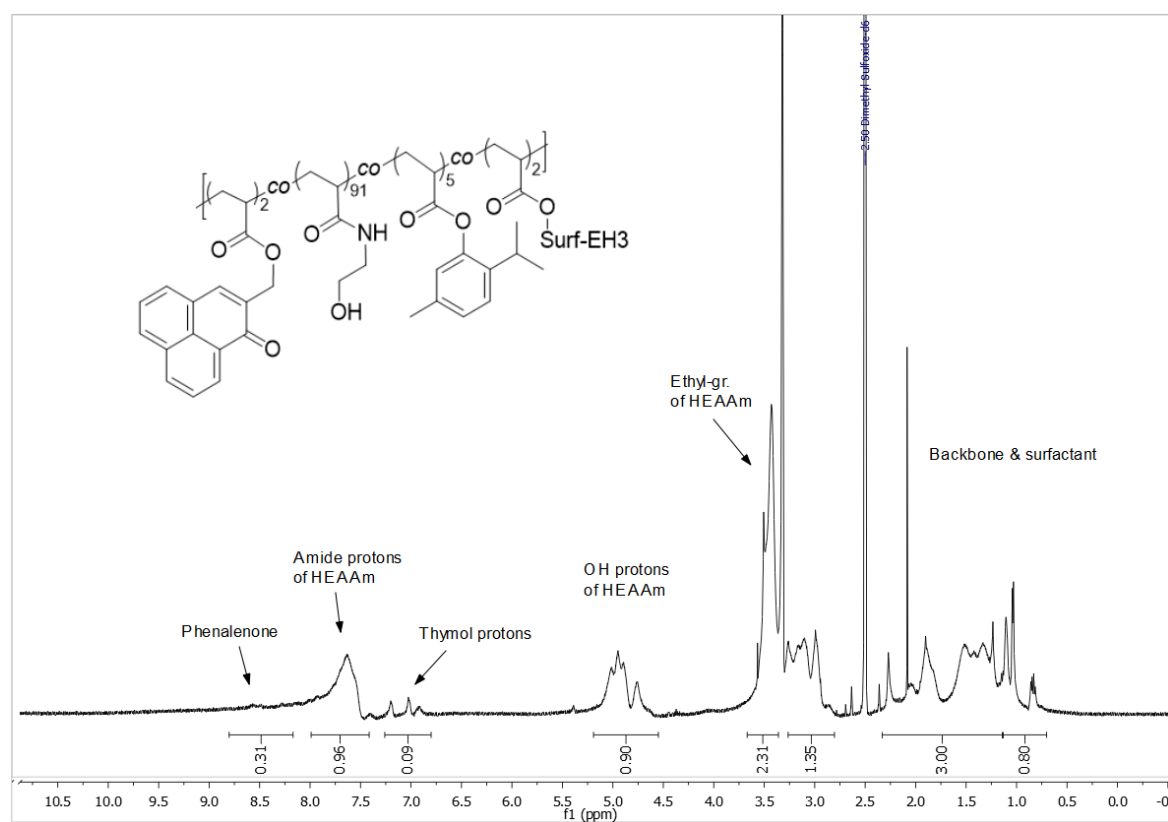
Fig. SI 41: ^1H NMR of $\text{poly}(\text{Ru}(\text{S})_1\text{-co-HEAAm}_{77}\text{-co-AA}_{20}\text{-co-Surf-EH3A}_2)$ (33).Fig. SI 42: ^1H NMR of $\text{poly}(\text{Ru}(\text{S})_1\text{-co-HEAAm}_{77}\text{-co-AOETAC}_{20}\text{-co-Surf-EH3A}_2)$ (33a).

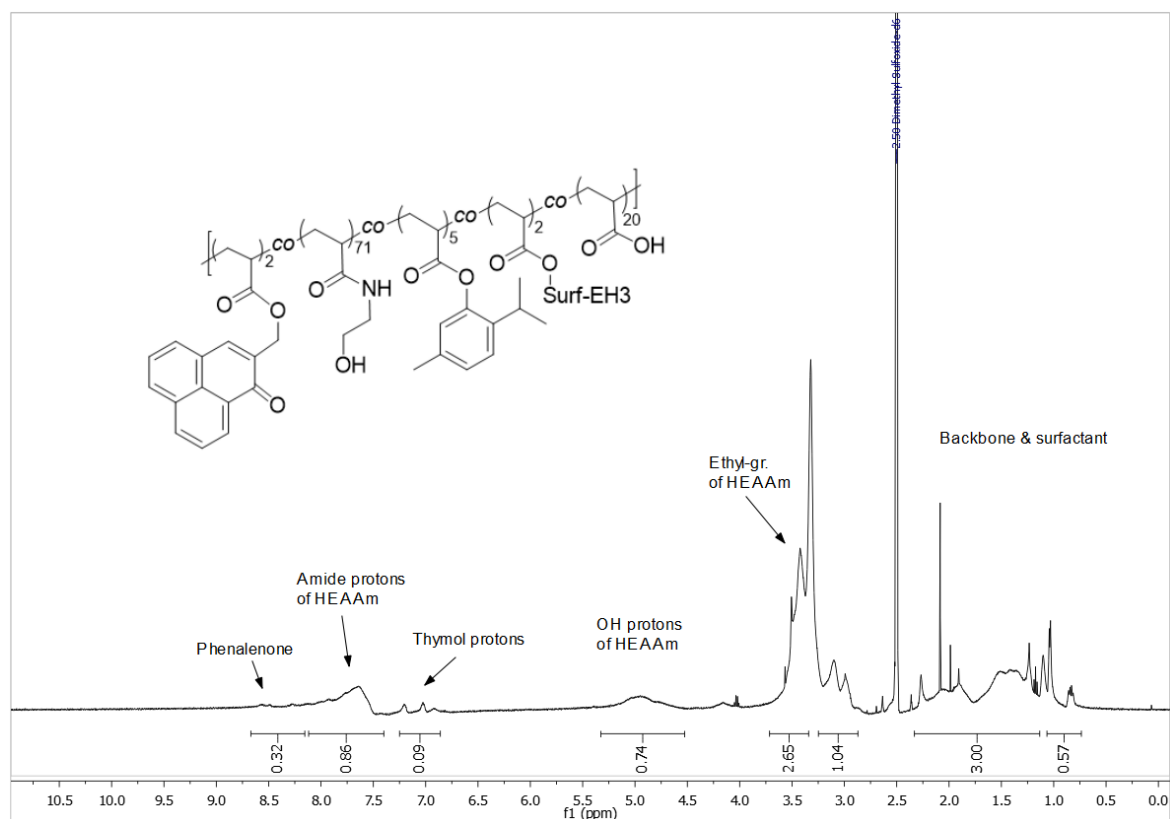
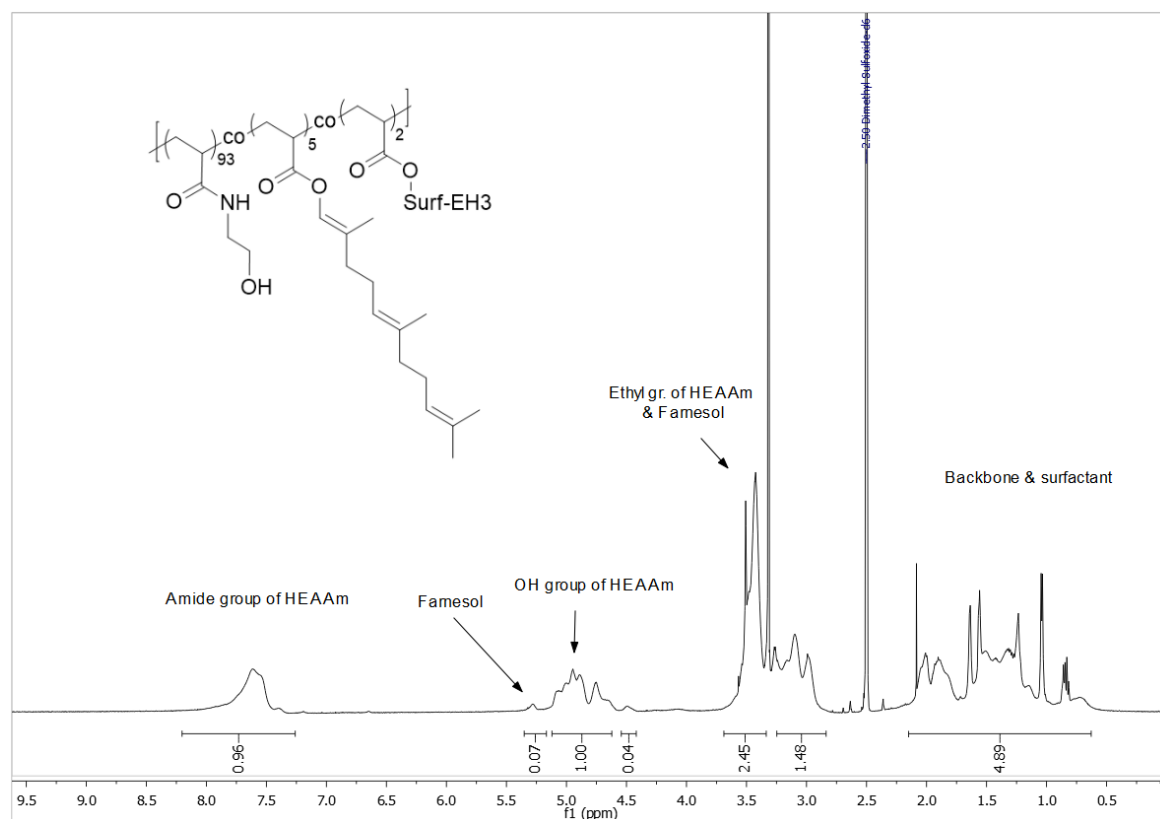
Fig. SI 43: ^1H NMR of poly(PN₂-co-HEAAm₉₆-co-Surf-EH3A₂) (35).Fig. SI 44: ^1H NMR of poly(Ru(AA)_{0.6}-co-HEAAm₉₅-co-Surf-EH3A_{4.4}) (36).

Fig. SI 45: ^1H NMR of poly($\text{Ru}(\text{AA})_{0.5}\text{-co-HEAAm}_{99.5}$) (**36a**).Fig. SI 46: ^1H NMR of poly($\text{PN}_2\text{-co-HEAAm}_{76}\text{-co-AA}_{20}\text{-co-Surf-EH3A}_2$).

Fig. SI 47: ^1H NMR of poly(PN₂-co-HEAAm₇₆-co-AOETAC₂₀-co-Surf-EH3A₂).Fig. SI 48: ^1H NMR of polyHEAAm₇₄-co-PN₂-co-Ru(S)₂-co-AA₂₀-co-SurfEH3A₂.

Fig. SI 49: ¹H NMR of S1.Fig. SI 50: ¹H NMR of S3.

Fig. SI 51: ¹H NMR of S4.Fig. SI 52: ¹H NMR of S5.

Fig. SI 53: ^1H NMR of S6.Fig. SI 54: ^1H NMR of Poly(Farnesol₅-co-HEAAm₉₃-co-Surf-EH3A₂) (40).

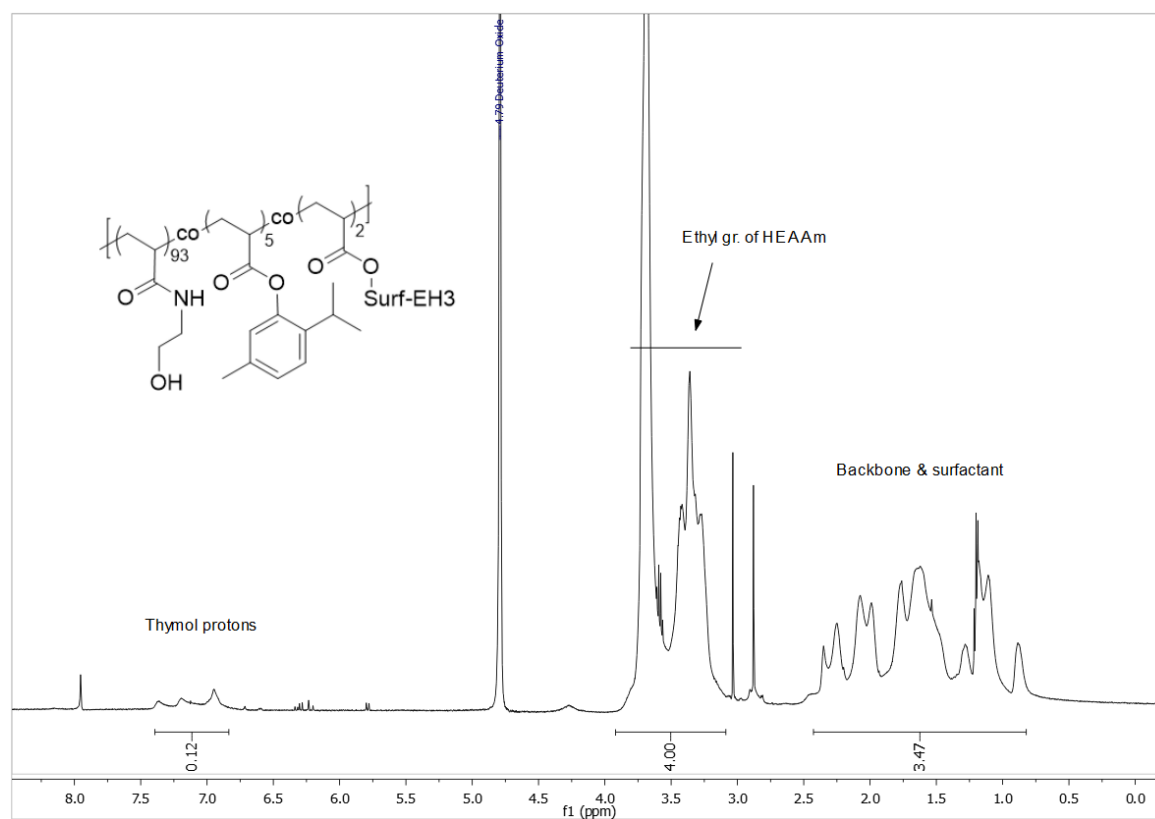


Fig. SI 55: ^1H NMR of Poly(Thymol₅-co-HEAAm₉₃-co-Surf-EH3A₂) (**41**).