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Synthesis of Biomimetic Phosphorus-Containing Polypeptides

Author
Yakovlev, Ilya

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Synthesis of Biomimetic Phosphorus-Containing Polypeptides

A dissertation submitted in partial satisfaction of the requirements of the degree Doctor of Philosophy in Chemistry

By

Ilya Yakovlev

2014

ABSTRACT OF THE DISSERTATION

Synthesis of Biomimetic Phosphorus-Containing Polypeptides

by

Ilya Yakovlev

Doctor of Philosophy in Chemistry

University of California, Los Angeles, 2014

Professor Timothy J. Deming, Chair

Immune response has historically been a major issue in the drug delivery field. In order to combat this problem, drug carriers have been designed to avoid the body's innate defenses. Following decades of research, it was found that an ideal drug delivery vehicle should enter the body, evade the immune system, stay in circulation long enough to seek out affected cells, and release the payload selectively. Furthermore, the vehicle should be comprised of materials that are non-toxic and biodegradable. While a number of potentially viable materials have been produced, there has arguably not been one which encompasses all these properties.

Using phosphocholine-containing vesicles or micelles as drug delivery vehicles is one potential solution. Phospholipids, the major components of biological membranes, have also been explored for drug delivery purposes; however, their use has not been successful due to the low stability of phospholipid vesicles. Thus, biomimetic polymers with pendant phosphocholine groups were used to increase drug delivery vehicle stability. Much like other polymers, polypeptides may serve as a superior alternative due to the biodegradability of their backbone.

Polypeptide synthesis has been thoroughly explored in the previous decades; however no reliable method for the preparation of phosphorus- and phosphatidylcholine-containing polypeptides has been published by the inception of this work.

Herein we discuss methods for preparation of phosphonate and phosphatidylcholine containing polypeptides. Synthesis of these materials builds on robust methods often employed in solution phase DNA synthesis. Protecting group strategies as well as the failures leading up to the polymers' successful synthesis are described. The polymers are synthesized via $Co(PMe_3)_4$ initiated polymerization of NCAs and are incorporated into diblock copolypeptides. The resultant products display interesting solution and calcium-binging properties.

The dissertation of Ilya Yakovlev is approved

Yves Rubin

Gerard C. L. Wong

Timothy J. Deming, Committee Chair

University of California, Los Angeles

2014

For my parents, Elena and Andrey Yakovlev

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LIST OF ABBREVIATIONS

a.u.	arbitrary units
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
br s	broad signal (NMR)
ca.	circa
Cbz	Carboxybenzyl
CD	Circular Dichroism
d	doublet (NMR)
DCM	Dicholomethane
dd	doublet of doublets (NMR)
DEPS	Diethylphosphoserine
DIPS	Diisopropylphosphoserine
DIPEA	Diisopropylethylamine
DLS	Dynamic Light Scattering
DMAc	Dimethylacetamide
DMF	Dimethylformamide
DMPS	Dimethylphosphoserine
DP	Degree of Polymerization
dr	diastereoselectivity
dt	doublet of triplets (NMR)
d-TFA	Deuterated Trifluoroacetic Acid
EPR	Enhanced Permeability and Retention

EtOAc	Ethyl Acetate
FDA	Food and Drug Administration
Fmoc	Fluorenylmethyloxycarbonyl
FTIR	Fourier Transform Infrared Spectroscopy
Glu	Glutamate
GPC	Gel Permeation Chromatography
Hex.	Hexane
Lys	Lysine
M:I	Monomer to Initiator Ratio
MeOH	Methanol
MHz	Megahertz
NCA	N-Carboxyanhydride
NMR	Nuclear Magnetic Resonance
Pal	Phosphonoalanine
PBS	Phosphate Buffered Saline
PC3	Human Prostate Cancer Cell Line
Pd/C	Palladium on Carbon
PDI	Polydispersity Index
PEG	Polyethylene Glycol
PEG1K-NCO	Isocyanate-Terminated Polyethylene Glycol, M_n=1000
PEG2K-NCO	Isocyanate-Terminated Polyethylene Glycol, M_n=2000
Ph	Phenyl
Pha	Phosphonohomoalanine

PMPC	Poly(2-methacryloyloxyethyl phosphorylcholine)
PXRD	Power X-Ray Diffraction
Pyr.	Pyridine
q	quartet (NMR)
rac	Racemic
ROP	Ring Opening Polymerization
S	singlet (NMR)
SEM	Scanning Electron Microscopy
Ser	Serine
SPPS	Solid Phase Peptide Synthesis
t	triplet (NMR)
tBu	*tert*-Butyl
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
TLC	This Layer Chromatography
TMS	Trimethylsilyl
UV	Ultraviolet
Z	Carboxybenzyl

Acknowledgements

I would like to thank my advisor, Dr. Tim Deming, for his support throughout my graduate career. He gave me the freedom to pursue my own ideas, experience failures, and achieve individual success. I would also like to acknowledge the excellent faculty at the UCLA Department of Chemistry and Biochemistry. Their knowledge and guidance allowed to me to grow both as a chemist and as a person. The people in my cohort have given me endless favors and help with new concepts and techniques. Namely, Tristin Rose has given me valuable advice, without which I would not have gotten through the toughest parts of my scientific journey.

The members of the Deming group were also instrumental in my success; they gave me input about my work and took the edge off when the going got tough. In particular, April Rodriguez turned from my labmate to one of my best friends; I will never forget our talks about chemistry, our trips to the gym, and our coffee breaks. I hope our friendship continues to grow.

I thank Linda Zarzana, Tammi Hong, and Dr. Kristin Casale at American River College in Sacramento for instilling me with an early love of chemistry, and my undergraduate advisor form University of California, Davis, Dr. Mark Mascal, for his mentorship and help with the decision to pursue a graduate degree. I will always remember Dr. Henning Stahlberg, whose wisdom and generosity allowed me not only to gain academic diversity, but to also learn how to balance my personal life with my career.

Finally, I wish to thank my family. My parents Elena and Andrey, and my sister Vera, have always given me unconditional support, but also constructive criticism when it was needed. I am forever grateful to my wonderful partner, Kim Phifer, who stuck with me through the reclusive phases as I was frantically completing my dissertation research. I couldn’t have done it without her.

CURRICULUM VITAE

Ilya Yakovlev

Education

Chemistry, Doctoral Candidate **September 2014**
University of California, Los Angeles, CA

Bachelor of Science in Biochemistry and Molecular Biology **June 2007**
University of California, Davis, CA

Professional and Academic Experience

University of California, Los Angeles **2009-present**
Graduate Student Researcher

Advisor: Dr. Timothy J. Deming
Synthesis of phosphatidylcholine and phosphonate containing polypeptide for biomedical research.

University of Basel, Switzerland **Summer 2011**
Graduate Student Intern

Advisor: Dr. Henning Stahlberg
Isolation and electron microscopy of E. Coli molecular rotor protein complexes

University of California, Davis **2007-2008**
Junior Specialist

Advisors: Dr. Paul Fitzgerald and Dr. Henning Stahlberg
In vitro assembly of protein filament and imaging via cryo-electron microscopy

University of California, Davis **2005-2007**
Undergraduate Researcher

Advisor: Dr. Mark Mascal
Synthesis of a fluoride-selective molecular host

Teaching Experience

Teaching Assistant at UCLA **2008, 2012-2014**
Courses: general chemistry, organic chemistry, biochemistry, bioconjugates.

Honors

UCLA Chemistry and Biology Interface Fellow **2009-2012**

UC Davis LaRock Undergraduate Research Conference, First Place **2007**

Publications

Yakovlev, I.; Deming, T.* "Synthesis of Poly(L-phosphatidylcholineserine) and its Properties." *In preparation*

Yakovlev, I.; Deming, T.* "Directing the Formation of Calcium Carbonate Microspheres Using Poly(L-phosphoserine) Analogs." *In preparation*

Yakovlev, I.; Deming, T.* "Analogues of Poly(L-phosphoserine) via Living Polymerization of Phosphonate-Containing N-Carboxyanhydride Monomers" *ACS Macro Lett.* **2014**, *3,* 378-381.

Mascal, M.*; **Yakovlev, I.**; Nikitin, E. B.; Fettinger, J. C. "Fluoride-Selective Host Based on Anion–π Interactions, Ion Pairing, and Hydrogen Bonding: Synthesis and Fluoride-Ion Sandwich Complex." *Angew. Chem. Int. Ed.* **2007**, *46,* 8782-8784

CHAPTER ONE: Introduction

1.1 Drug Delivery

Traditional drug delivery methods rely on the body's intrinsic distribution system for transfer of drugs to areas of interest. Oral ingestion, injection, or aerosol inhalation, are often employed as administration methods. While such methods have been very successful for centuries, they suffer from low efficiency because much of the drug is unused for its intended purpose. These problems stem from the body's constant effort to clear the drug and are further exacerbated by bioavailability issues. Furthermore, delivery to untargeted regions of the body leads to unwanted consequences such as inadvertent accumulation of drugs in the liver and spleen.

The drug delivery conundrum is perhaps most discussed in the area of cancer treatment. Of the numerous cancer drugs available, most cause severe side-effects, which lead to narrow therapeutic indexes. The difficulty of targeted cancer drug delivery can be attributed to the biochemical similarity of cancer cells to healthy cells. In an attempt to circumvent this problem, many small molecule drugs take advantage fast metabolism of cancerous tissue as a means of targeting. Although this approach has been effective to an extent, the resultant drugs still target healthy cells with intrinsically fast metabolic rates. To better target cancer cells specifically, an increasing amount of research has been focused on the development of drug delivery vehicles. Drug delivery vehicles, which target specific areas of the body, are desirable because of their ability to transport drugs more accurately as well as to possibly increase bioavailability.

The ideal drug delivery vehicle should enter the body, evade the immune system, stay in circulation long enough to seek out afflicted cells, and ultimately release the payload selectively to cancer sites. This set of guidelines is known as the "magic bullet," due to the envisioned

drug's ability to seek out affected tissue. A promising lead toward meeting this goal was reported by Yasuhiro Matsumura and Hiroshi Maeda in 1986.[1] The authors were performing studies with copolymers of styrene and maleic acid, conjugated to the antitumor protein nercarzinostatin. The original aim of their work was to use the diblock polymer in order to protect nercarzinostatin from degradation, increase its *in vivo* half-life, and enhance its lipophilicity. While they were delighted to find that their original goals were successful, they also noted that their conjugates were predominantly accumulating in tumor tissue. This accumulation was attributed to "leaky" vasculature and the lack of lymphatic drainage in solid tumors. Because of these properties the accumulation was called the "Enhanced Permeability and Retention" effect (EPR Effect).

Further characterization of cancer development in subsequent years showed that once a new cluster of cancerous cells reaches a diameter of 2-3nm, it recruits new blood vessels to the tumor site in order to continue growth.[2] The emerging neovasculature is vastly different from normal tissue: the blood vessels possess irregular shape and have large fenestrations. Furthermore, the surrounding smooth-muscle layer is abnormal or entirely absent.[2,3] These vascular defects, along with slow venous return and poor lymphatic drainage preferentially funnel and retain macromolecules into the tumor interstitium.[4] Macromolecules can accumulate at the tumor site in concentrations of 10-50 times higher than elsewhere in the body within one to two days (Figure 1.1).

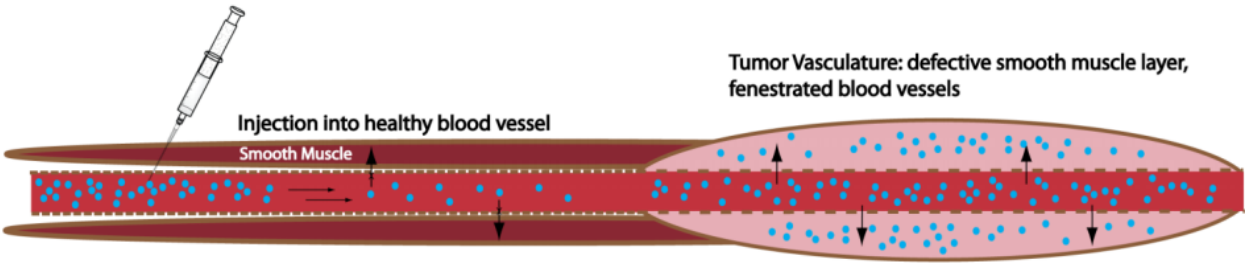


Figure 1.1. Tumor accumulation of macromolecular pharmaceuticals.

The EPR effect only applies to high molecular weight drugs, as their small molecule counterparts localize in the body indiscriminately and are cleared from circulation much more rapidly.[5] In addition to molecular weight limitation, macromolecular drugs must between 10 and 200 nm to display optimal tumor accumulation.[6] Molecules with diameters below 10 nm are rapidly cleared by the kidneys,[7,8] and materials above 200 nm do not efficiently permeate the leaky tumor vasculature, and thus stay in circulation.[1,9] Because of these promising properties, research on macromolecular pharmaceuticals has exploded over the past two decades, and a number of new macromolecular formulations of already existing small molecule drugs have entered clinical trials.

1.2 Phospholipids and Polymers in Drug Delivery

Phospholipids are amphiphilic molecules which are capable of self-assembly into micelles, vesicles, and other structures in aqueous solution. Due to their hydrophilic-hydrophobic properties, these amphiphiles can form dynamic membranes on the nano scale. Synthetic phospholipids can be prepared to create artificial membranes for drug delivery applications. These membranes can form vesicles or other aggregates which can act as nanocompartments for drug delivery.[10] In order to mimic the properties of phospholipids, polymers bearing block architectures have been prepared, which contain hydrophilic and hydrophobic segments. These hydrophilic-hydrophobic motifs result in amphiphilic materials, similar to those of phospholipids (Figure 1.2).

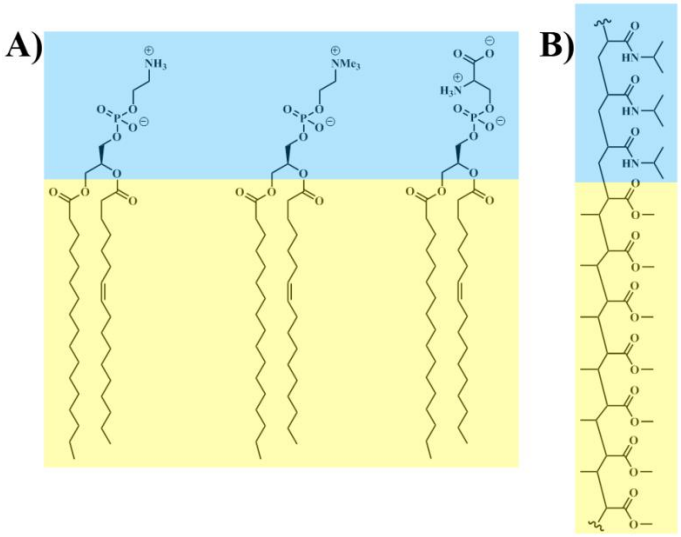
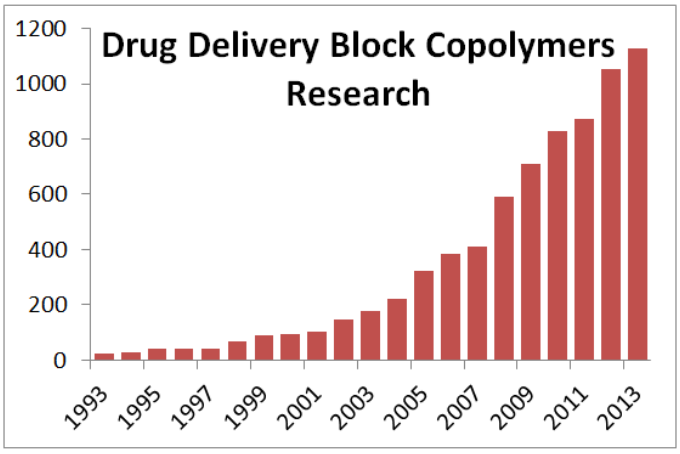


Figure 1.2. Examples of amphiphilic phospholipids (A), and an amphiphilic polymer (B). The blue area represents hydrophilic regions; the yellow area represents hydrophobic regions.

Block copolymers can self-assemble into a number of diverse morphologies such as helices,[11,12] torroids,[13] spheres,[14,15] rods,[16,17] discs,[18] fibers,[19,20] tubes,[21,22] vesicles,[23-26] and micelles.[27-30] Due to their diversity and versatility, block copolymers have generated a substantial amount of scientific activity in the last two decades; an ISI web of Knowledge search for "drug delivery block copolymers" shows a near 3-order of magnitude increase in publications from 1993 to 2013. The increase can be partially attributed to a wide range of new polymer materials, allowing for greater control of self-assembly and related factors.[31,32]



1.3 Polymers Used in Drug Delivery

The ideal polymer-based drug delivery system, would achieve all of the following criteria: non-immunogenicity, high stability for prolonged circulation half-life, efficient drug loading and release, specific targeting, minimal or non-existent toxicity, and biodegradability. Care must also be taken to minimize cost, synthetic steps, and unwanted properties such as premature drug release.

To date, polyethylene glycol (PEG) has been the most commonly used polymer in FDA approved drug delivery systems.[33] Its popularity stems from its low toxicity and its ability to evade the immune system. Drugs used in cancer medicine are predominantly hydrophobic and consequently have inferior solubility profiles. In order to increase the solubility of hydrophobic drugs, they can be complexed with hydrophobic carrier polymers, and then the assemblies can be coated with PEG. This approach has been coined "pegylation," a term which finds near daily use in the polymer chemists' vocabulary. Figure 1.3 shows some examples of pegylated drug delivery systems.

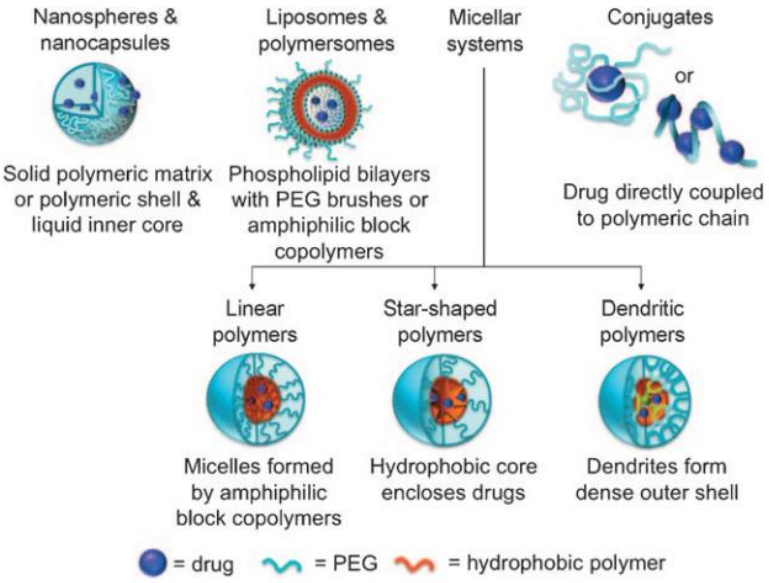


Figure 1.3. The use of PEG in drug delivery systems.[33]

One drawback of using PEG is that the repeat using cannot be functionalized. The lack of functionality restricts chemistry that can be performed to ends of the polymer, which is a severe restraint to the design of extra features, such as targeting or stimulus response. Another problem with PEG is the polymer's non-biodegradability, which limits the molar mass of individual polymers usable *in vivo* to 20-60kDa.[34-36]

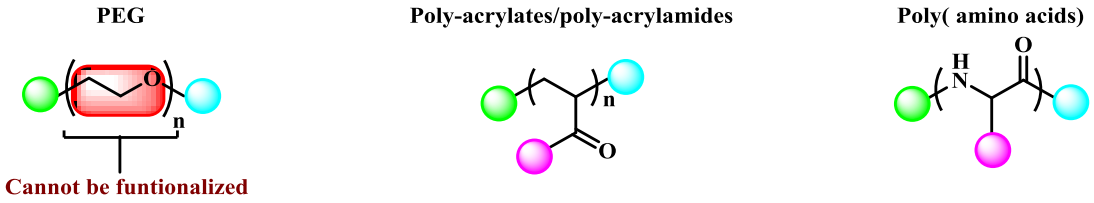


Figure 1.4. PEG vs other polymers. Spheres represent sites that can be functionalized.

The functionalization problem has been addressed by synthetic PEG alternatives. Notable examples include poly(amino acids)[37-39], poly-acrylates and poly-acrylamides,[40,41] polyesters,[42] and others.[43]

1.4 Polypeptides

Polypeptides have become increasingly popular in drug delivery research. Mimicking nature, synthetic polypeptides can be comprised of the 20 common natural amino acids, or be made from modified amino acids. One benefit of using polypeptides is the inherent biodegradability of the polymer backbone. This feature allows researchers to design polymers of higher individual mass relative to their non-biodegradable counterparts.

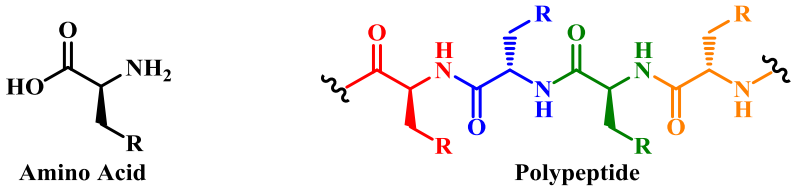


Figure 1.5. Amino acids and polypeptides variable at R-groups.

The secondary structure of polypeptides, which stems from chirality of amino acids, is another useful feature. Secondary structures of polypeptide polymers are further stabilized by hydrogen bonding, and can be used when constructing macromolecular species. Furthermore, polypeptides can respond to stimuli such as redox triggers,[44,45] pH,[46-49] and temperature.[50-52]

1.5 Synthesis of Polypeptides

Synthetic polypeptides are prepared using two main strategies: solid phase peptide synthesis (SPPS) and polymerization. SPPS is a stepwise approach, which uses resin-bound starting materials to add amino acids one by one.[53]

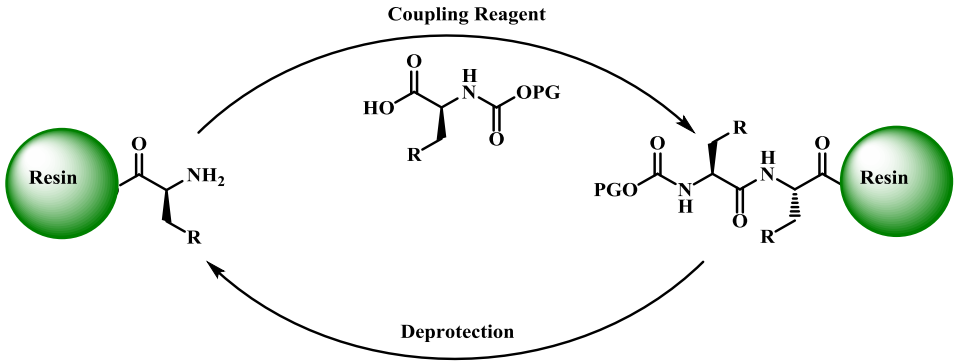


Figure 1.6. Solid phase peptide synthesis, PG = protecting group.

Typically, SPPS couples Fmoc-protected amino acids onto the resin using a peptide coupling reagent. Following attachment, the protecting groups are removed, which liberates the amino termini and allows for subsequent addition of amino acids. The stepwise synthesis is both slow and expensive. For example, the production of 100 mg of a 20 amino acid polypeptide uses many

of solvents and may take over 24 hours to complete.[54] Furthermore, large polypeptide segments become very difficult to synthesize due to small inefficiencies in each step, which compound with longer syntheses. As a result, this method is generally not used for production scale.

Ring opening polymerization (ROP) of N-carboxyanhydrides (NCAs) is another widely used method for polypeptide preparation. This method has been known for over a century, but has only recently gained traction as a viable technique for preparation of well-defined polypeptides.[55] NCAs are typically prepared via two methods: chlorination of carbamate-protected amino acids (Leuchs method),[56,57] or phosgenation of free amino acids.[55]

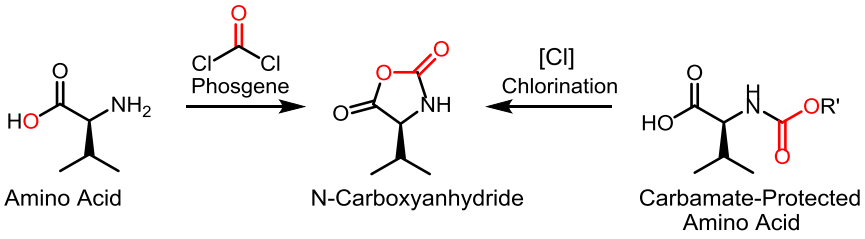


Figure 1.7. Preparation of NCAs

The resulting highly-strained 5-membered ring of NCAs is highly susceptible to nucleophilic attack; therefore most polar side chains must be protected. Using inert atmosphere glove boxes and dry reaction conditions are precautions taken by most chemists who work with NCAs.

1.6 Polymerization of NCAs

Early NCA polymerization methods have traditionally had limited success due to numerous side reactions and low yields of target materials.[55] However, amine-initiated NCA polymerizations have seen some success. The amine-initiated polymerization starts by a nucleophilc attack on the NCA by an amine, which opens the NCA ring and generates a carbonic acid. Carbon dioxide is then liberated, regenerating the amine. The new amine then propagates the chain by repeating the process (Figure 1.8).

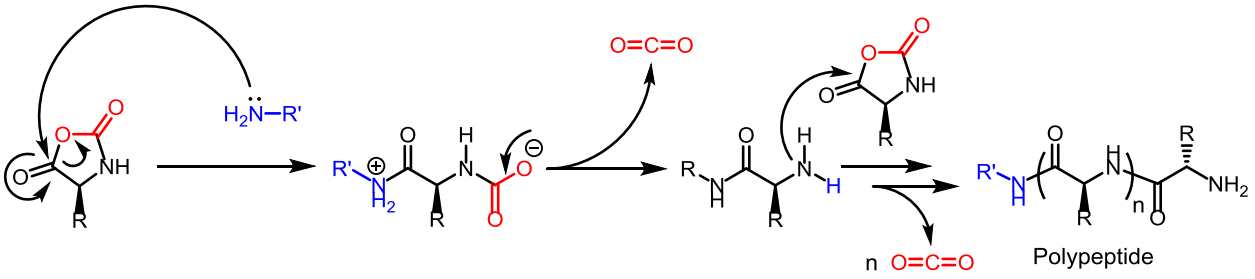


Figure 1.8. Amine-initiated NCA polymerization.

Unfortunately, this approach is prone to side reactions, which lead unwanted byproducts including as oligopeptides or cyclic peptides.[58] Recently, silylated amines were used to reduce such side reactions,[59,60] although the adoption of this method has not been widespread.

1.7 Transition Metal Mediated Polymerization of NCAs

Prior to the work in this dissertation, the Deming lab established a new NCA polymerization method. Using zerovalent cobalt and/or nickel initiators, developed in house, the lab was able to polymerize NCAs to full conversion with excellent chain length control (Figure 1.9). Furthermore, this method produced homopolypeptides and block-copolypeptides of high molecular weights and low polydispersities.[61-63]

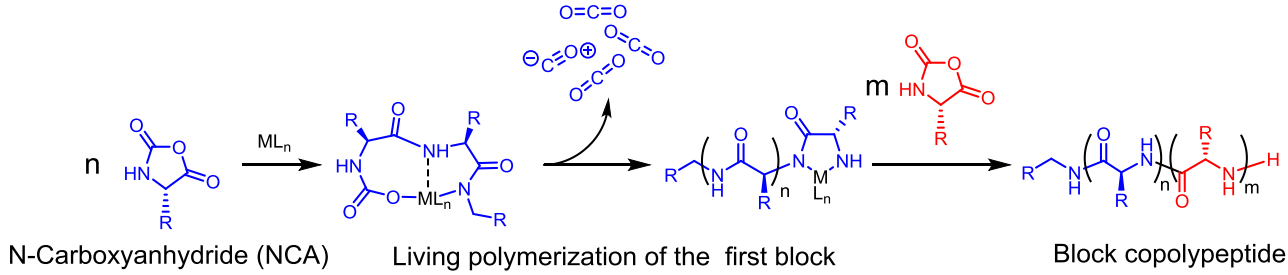


Figure 1.9. Zerovalent cobalt- or nickel-mediated polymerization of NCAs

1.8 Block Copolypeptides

Much like other polymers used in drug delivery research, polypeptides can self-assemble into a wide range of structures. Polypeptides-comprised drug delivery vehicles of various morphologies have been explored, of which many rely on block copolypeptides' ability to form artificial membranes. To date, the Deming lab has developed a number of block copolypeptide assemblies, most of which follow the membrane-forming, hydrophilic-hydrophobic motif. The hydrophobic block typically functions as a structural component of artificial membranes and dictates the size and shape of the assemblies. The hydrophilic block is essential for solvation, stimuli response, and interactions with biological systems. As such, the hydrophilic block must have low cytotoxicity, sufficient solubility, and non-immunogenicity.

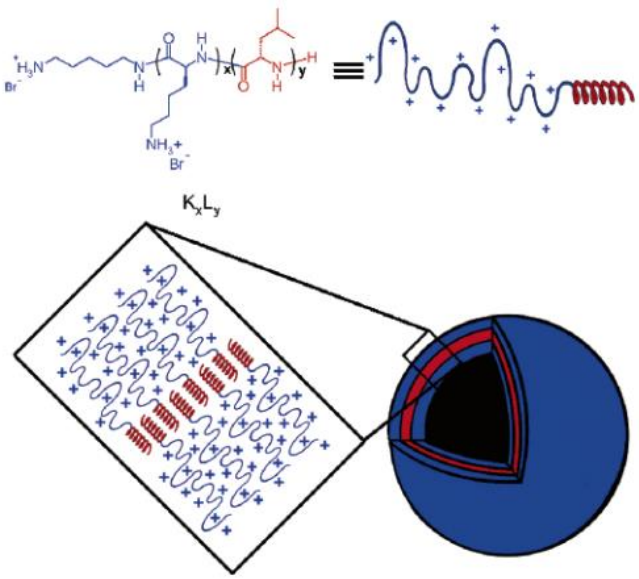


Figure 1.10. Polylysine-*block*-polyleucine vesicles.[64]

First generation materials prepared in the Deming lab used poly-lysine or poly-glutamate as hydrophilic blocks. Both were shown to self-assemble into vesicles with sizes in the EPR range (Figure 1.10); however lysine-containing vesicles were toxic and glutamate containing vesicles could not enter cells effectively, although they were non-toxic. These results were in agreement

with previous research, which showed that highly cationic materials enter cells readily, but display cytotoxicity.[65,66] Anionic materials generally do not suffer from cytotoxicity problems, but also do not efficiently enter cells. In order to combat these problems, recent research has focused on methionine-based polypeptides. Methionine blocks can be oxidized or modified,[67,68] and show promising results in both cytotoxicity and cellular uptake studies. It is presently unclear what the ideal block copolypeptide will look like, however it will need to meet the aforementioned requirements to be an ideal drug delivery material.

CHAPTER TWO: Phosphono-Polypeptides

2.1 Introduction

Phosphate-containing polymers have attracted recent attention for their utility in a wide range of applications, including dental adhesives, adhesion promoters, ion exchange resins, and flame retardants.[69] For medical uses, such as bone regeneration, it would be advantageous for these polymers to mimic the features of natural phosphoproteins, including bioactivity and biodegradability.

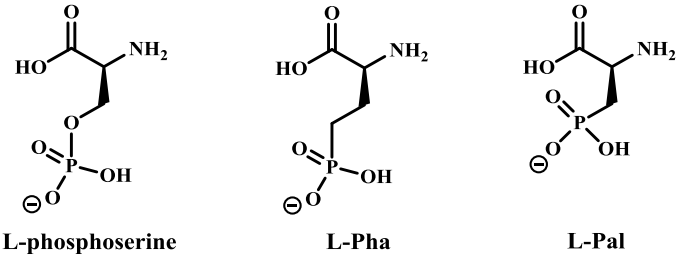


Figure 2.1. Structures of phosphoserine and phosphonate analogs

Phosphoserine (Figure 2.1) is a key component of phosphoproteins that mediate diverse processes such as protein-protein interactions,[68] protein activation and inhibition,[70] and biomineralization.[71] Serine-rich osteopontin has been shown to nucleate hydroxyapatite deposition, but only when highly phosphorylated.[72]

Synthetic polypeptide mimics of phosphoproteins have been studied for many years, yet production has been limited by inefficient synthesis and difficulty with incorporation into well-defined block copolymers.[73,74] To mitigate these issues, we synthesized new phosphonate containing α-amino acid N-carboxyanhydride (NCA) monomers, which were to prepare well-defined phosphonate containing polypeptides and block copolypeptides. These NCAs were

found to undergo living polymerization to high molecular weights with narrow chain length distributions. One polymer, poly(*L*-phosphonohomoalanine), poly(Pha), is an isosteric analog of poly(*L*-phosphoserine) and was found to display a pH responsive conformational change.

Homopolymers of L-phosphoserine have been prepared via polymerization of diphenylphosphoserine NCA.[73] Removal of protecting groups also required an expensive catalyst, lessening the practical utility and applicability of this approach. The poly(L-phosphoserine) obtained was found to be conformationally disordered over a pH range of 1.3 to 12. Recently, new potential mimics of poly(L-phosphoserine), based on phosphate and phosphonate containing S-alkyl-L-cysteine derivatives, were prepared from the corresponding functionalized NCA monomers.[74] In this system, phosphorous containing homopolypeptides could be prepared with controlled chain lengths up to degrees of polymerization of 40, and with defined C-terminal end-groups. However, the phosphorylated polypeptide could not be deprotected without dephosphorylation, and no block copolypeptides were prepared.[74] The polyphosphonate derivative could be deprotected, and gave a water soluble homopolypeptide with a disordered conformation at pH 7.2. One key feature of these polypeptides is the length of

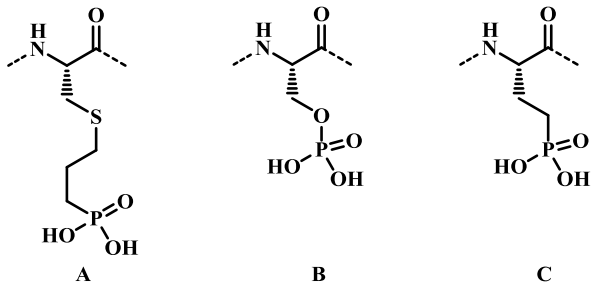


Figure 2.2. Phosphoserine analogs. (A) Synthesized by Gupta et. al.;[74] (B) Phosphoserine; (C) Phosphonohomoalanine.

their side chains, which are significantly longer than in phosphoserine (5 versus 2 atoms between backbone and phosphorous, respectively), which may limit their ability to mimic natural

phosphorylated proteins (Figure 2.2). Our lab became interested in preparation of phosphoserine analogs, especially for incorporation into self-assembling block copolypeptides for biomaterials uses.[39]

2.2 Synthesis and Polymerization of Dimethylphosphoserine NCA

Initial stages of this project focused on the development of a more direct route toward the preparation of phosphoserine NCA. As with all NCAs, we had to choose proper protecting groups for the side chain. The protecting groups needed to meet the criteria of being robust enough to survive synthetic manipulations prior to the polymer stage, and labile enough for easy removal. Furthermore, any other protecting groups used in the synthesis could not have any cross-reactivity with the protected phosphate. The methyl group served as an attractive starting point as it is easily installed, is very stable, and can be removed using mild aqueous conditions.[75,76] Thus we began work on the synthesis of dimethylphosphoserine NCA (DMPS).

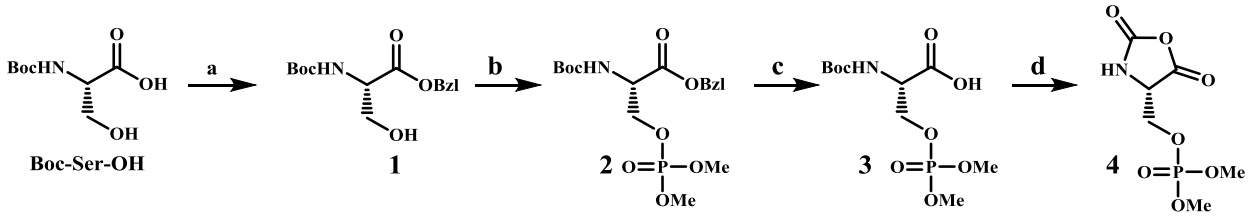


Figure 2.3. Synthesis of dimethylphosphoserine NCA. (a) BnBr, Et_3N, Acetone (95% yield); (b) PMe_3, pyr. CBr_4; (c) H_2, Pd/C (80% yield over 2 steps); (d) DCMME/DCM or Ghosez reagent (<20% yield).

The synthesis began with the commercially available Boc-Ser-OH, the α-carboxylate of which was protected using benzyl bromide in the presence of triethylamine. The protected phosphate group was introduced via oxidative phosphorylation,[77] followed by the removal of the benzyl protecting group from the α-carboxylate, providing **3** in excellent yield. Synthesis of the

NCA proved to be problematic due to low yields and purity. Efforts to purify the NCA via chromatography and/or crystallization were unsuccessful.

In the Deming lab, synthesis of NCAs via phosgenation has generally been preferred over Leuch's method due to a lower number of byproducts. DMPS synthesis was then attempted again using phosgene as the NCA forming agent (Figure 2.4). Starting with the previously synthesized commercially available Z-Ser-OBzl, oxidative phosphorylation followed by hydrogenolysis yielded clean NCA precursor **6**, which was elaborated to the NCA using

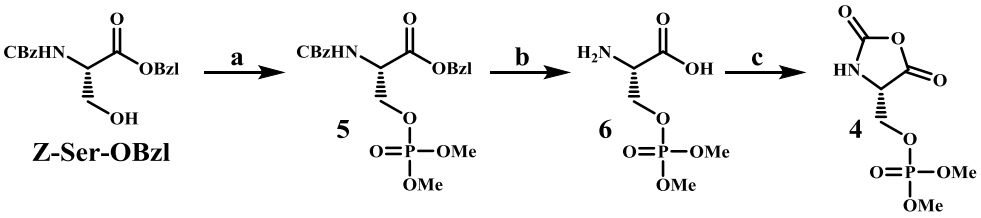
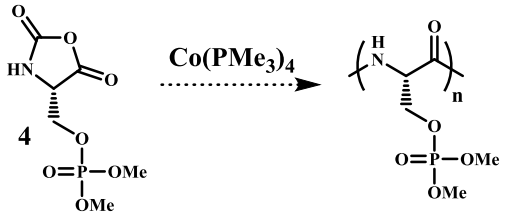


Figure 2.4. Alternate synthesis of dimethylphosphoserine NCA. (a) $P(OMe)_3$, pyr. CBr_4 (70-80% yield); (b) H_2, Pd/C (99% yield); (c) $COCl_2$, THF, 40°C (50% yield).

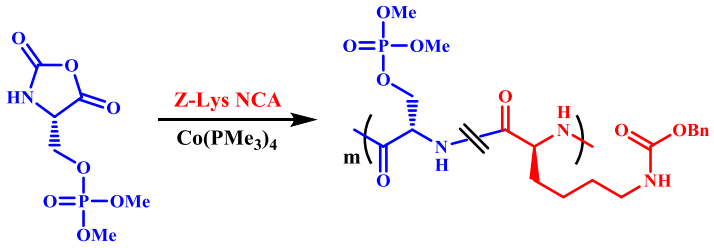
phosgene. The NCA was sparingly soluble in THF, which made the purification process very difficult and time consuming. As a result, a desirable level of purity was not achieved. Nevertheless, some polymerization experiments could still be attempted.



Solvent	Result
100% THF	Incomplete polymerization, insoluble products
50/50 THF/DMF	Incomplete polymerization, insoluble products
100% DMF	All NCA consumed, no polymer detected

Figure 2.5. Attempted polymerizations of dimethylphosphoserine NCA (4).

Polymerizations of DMPS using $Co(PMe_3)_4$ in THF gave insoluble material and did not go completion, reactions in mixtures of DMF and THF had similar results. Using 100% DMF as the solvent showed full conversion of the NCA, however, no polymer products could be detected via FTIR or GPC. We theorized that the methyl protecting groups did not impart sufficient solubility to DMPS polymers in organic solvents. Poly-serine derivatives are known for their poor solubility in literature,[78] presumably owing to significant intra and inter chain hydrogen bonding.



M:M:I[a]	Solvent	Polymerization Result
10:10:1	DMF	Incomplete
20:20:1	DMF	Incomplete
30:30:1	DMF	Incomplete
6:20:1	DMF	Incomplete
4:20:1	DMF	Complete, precipitate
2:20:1	DMF	Complete, no precipitate

Figure 2.6. Random copolymer synthesis attempts. (A) [DMPS]:[Z-Lys]:[$Co(PMe_3)_4$] ratios.

Random copolymer syntheses with Cbz-Lysine NCA (Z-Lys)[79] were attempted in order to increase the solubility of the overall polypeptide. Copolymers of varying monomer to initiator ratios were explored in order to gauge the minimum amount of DMPS that can be present within polymer before it becomes insoluble; the results are summarized in Figure 2.6. Polymerizations go to completion in THF when no more than 10 mol. % of DMPS vs Z-lysine NCA is used. While the PDI for that polymer was acceptable (M_w/M_n=1.17), the utility of such restrictive conditions is limited.

2.3 Synthesis and Polymerization of Diethylphosphoserine NCA

Difficulties in polymerization compounded with a rather troublesome synthesis of DMPS prompted us to consider alternative protecting groups. Following the aforementioned experiments, it became clear that we needed to improve the solubility of the NCA. We theorized that increasing hydrophobicity of the protecting group we may disrupt the aggregation caused by hydrogen bonding. We chose the ethyl group due to the wide availability of triethylphosphite, a reagent which may be used to synthesize diethylphosphoserine NCA (DEPS).

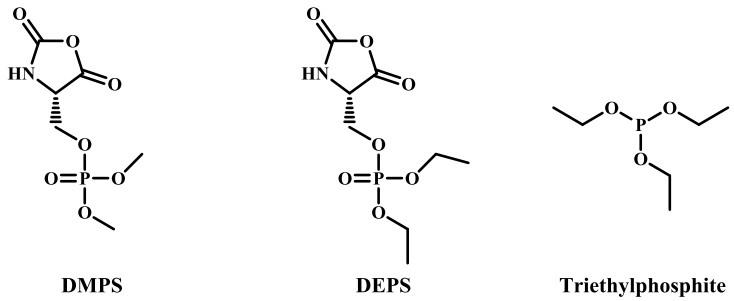


Figure 2.7. The increased hydrophobicity of the ethyl group versus the methyl group.

After optimization, it was found that the first route employed in DMPS synthesis was more suitable for the preparation of DEPS. The synthesis started with the previously prepared

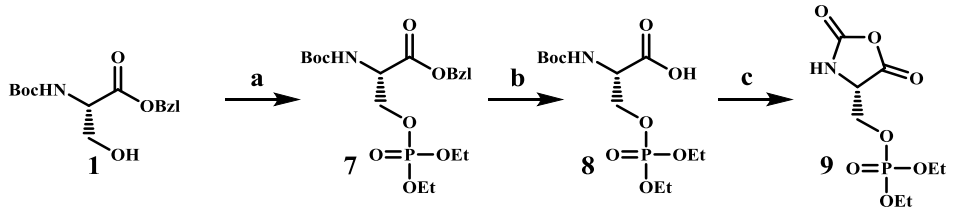
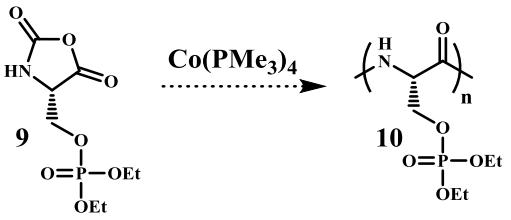


Figure 2.8. Synthesis of diethylphosphoserine NCA. (a) $P(OEt)_3$, CBr_4, Pyr. (78% yield); (b) H_2, Pd/C (90 % yield); (c) Ghosez reagent, THF, 18h (62% yield).

1, which was elaborated to **7** using oxidative phosphorylation. The α-benzyl protecting group was then removed via hydrogenolysis. NCA synthesis was accomplished using Ghosez reagent (Figure 2.8).[80]

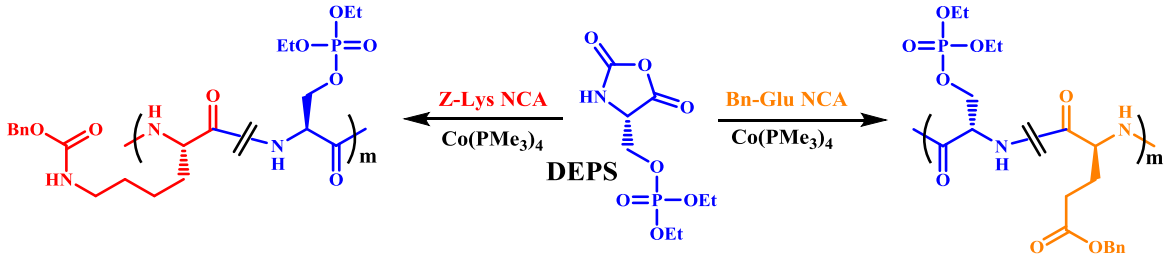
Polymerizations in THF did not go to completion and contained monomer at moderate [M]/[I] ratios. Polymerization attempted in DMF and DMAc gave similar results (Figure 2.9).



[M]/[I] Ratio	Solvent	Result
10:1	THF	Complete, Insoluble
25:1-75:1	THF	Incomplete, Insoluble
20:1-60:1	DMF	Incomplete, Insoluble
20:1-60:1	DMAc	Incomplete, Insoluble

Figure 2.9. Attempted polymerizations of DEPS.

These studies made it clear that DEPS would not yield a polymer with desired solubility properties. Random copolymerizations were carried out to gauge if we have achieved an improvement over DMPS in terms of solubility. This was tested by making a series of random copolymers with Z-lysine NCA or Bn-Glu NCA;[79] the results are summarized in figure 2.10.



M:M:I[a]	Second Monomer	M_n (x10^{-3} g*mol^{-1})	M_w/M_n
10:10:1	Z-Lysine	18.5	1.14
15:15:1	Z-Lysine	17.9	1.25
20:20:1	Z-Lysine	21.6	1.14
10:10:1	Bn-Glu	20.5	1.20
15:15:1	Bn-Glu	26.9	1.17
20:20:1	Bn-Glu	74.2	1.17

Figure 2.10. Random copolymerizations of DEPS with Z-Lys and Bn-Glu. M_n values can only be compared in relative terms as the *dn/dc* values for the polymers were not determined. [a] [DEPS]:[Z-Lys]:[$Co(PMe_3)_4$]ratios.

Random copolymers of varying lengths could be formed with some level of length and polydispersity control when a 50/50 mixture of DMF and THF was used as solvent. Indeed, our assertions about increasing solubility relative to the hydrophobicity of the protecting group were correct, however not to the extent that we had hoped.

2.4 Synthesis and Polymerization of Diisopropylphosphoserine NCA

Building on improvements in solubility observed with the ethyl protecting group, we decided to increase the hydrophobicity of the protecting group yet again. We chose to use isopropyl as it has one more carbon than ethyl, and can be made using the inexpensive and readily available triisopropylphosphite. We hoped that the isopropyl group would impart sufficient solubility within diisopropylphosphoserine NCA (DIPS) and its homopolymers (Figure 2.11).

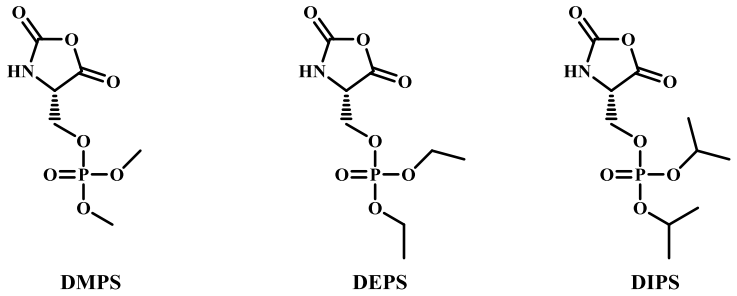


Figure 2.11. Structure of dialkylphosphoserine NCAs.

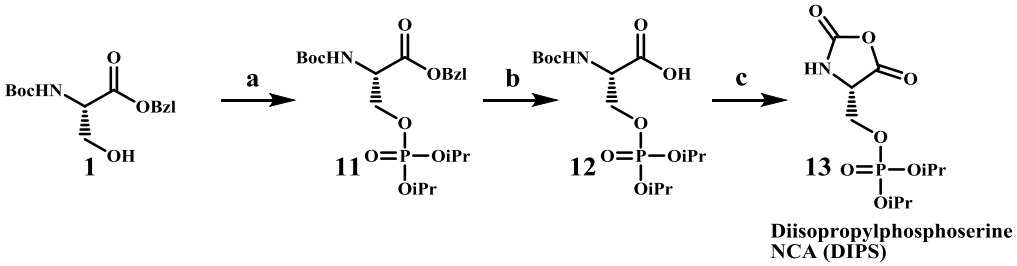


Figure 2.12. Synthesis of DIPS. (a) $P(OiPr)_3$, pyr. CBr_4 (90% yield); (b) H_2, Pd/C (84% yield); (c) Ghosez reagent, THF, 18h (43% yield).

DIPS was synthesized via Leuchs method using a Boc protecting group on the α-amine. We used the route that was utilized in the synthesis of DMPS (Figure 2.12). The NCA was obtained in good yield as an off-white solid. It is worth noting that as the bulk of the alkyl groups increased so did the ease of synthesis. The amount of insoluble and highly colored byproducts went from considerable with the methyl group, to manageable with the ethyl group, to nearly non-existent with isopropyl. Furthermore, this was the first NCA in the dialkylphosphoserine series to be isolated as a solid.

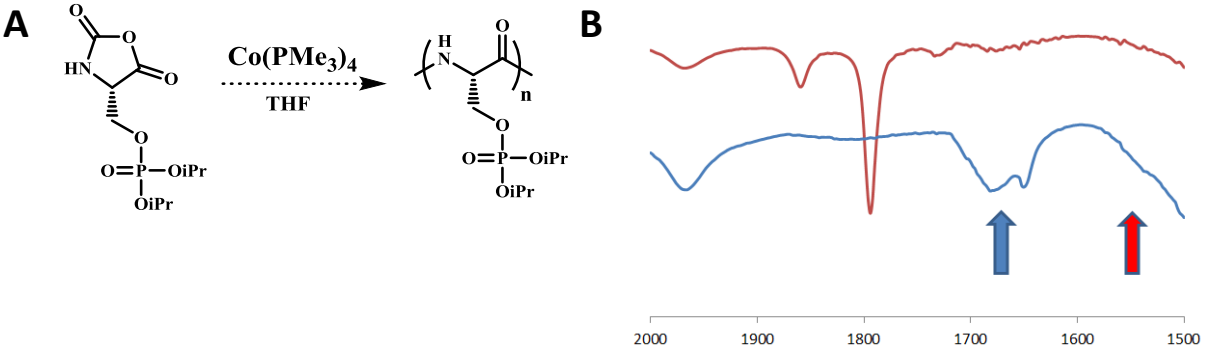
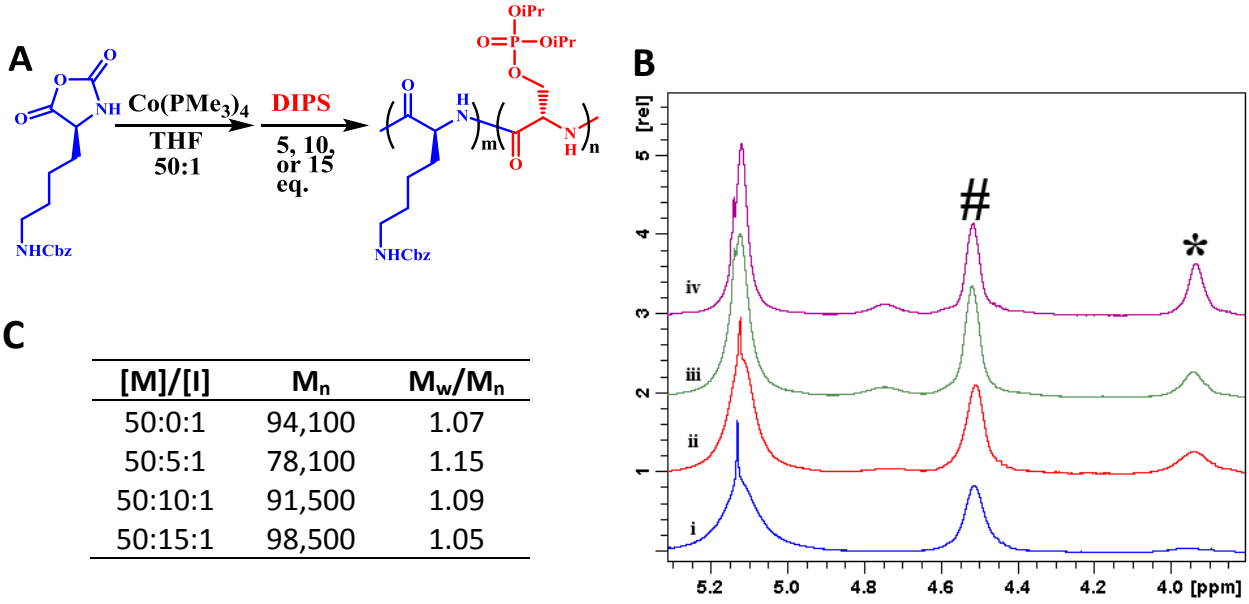


Figure 2.13. (A) Polymerization of DIPS. (B) FTIR spectrum of DIPS in THF (red, top); FTIR spectrum of polymerization product (blue, bottom), signal at 1650-1690 cm^{-1} (blue arrow); absence of signal at 1550 cm^{-1} (red arrow).

DIPS was fully consumed in THF at [M]/[I] ratios of up to 20:1 with no insoluble byproducts. The polymerization products did not show the typical α-helical polypeptide FTIR

stretches at 1650 cm^{-1} and 1540 cm^{-1} but instead displayed 2 overlapping bands from 1650 ~ 1690 cm^{-1}, and showed no signal at 1540 cm^{-1} (Figure 2.13). Furthermore, the polymer gave no GPC signal using LS or RI detection. Diblock copolymers with Z-Lysine NCA were prepared in order to further characterize the polymerization properties of DIPS. We chose to synthesize a long poly-Z-lysine block and grow short segments of poly-DIPS off of it for both NMR and GPC analysis. Since poly-Z-lysine has been very well characterized in the past, any deviation from the expected lysine homopolymer would be easily noticed. A lysine block at a [M]:[I] ratio of 50:1 was synthesized, followed by addition of 5, 10, or 15 equivalents of DIPS.



C

[M]/[I]	M_n	M_w/M_n
50:0:1	94,100	1.07
50:5:1	78,100	1.15
50:10:1	91,500	1.09
50:15:1	98,500	1.05

Figure 2.14. (A) Scheme for the synthesis of Z-Lys-*block*-DIPS block copolypeptides. (B) ^{1}H NMR data in *d*-TFA showing different lysine to DIPS compositions. Peaks labeled # are the CH_2NH resonances of Z-lys while * is the OCH resonance of DIPS; i = 50:0:1; ii = 50:5:1; iii = 50:10:1, vi = 50:15:1. (C) GPC results for diblocks. Ratios represent molar amounts of Z-Lys first block to DIPS second block to $Co(PMe_3)_4$ initiator.

Due to unknown *dn/dc* values of these diblock copolypeptides, GPC data may only be compared qualitatively. M_n values increase with each elongation of the second block, which is consistent with increases in lengths of DIPS segments. Furthermore, NMR data shows a clear, proportional increase in the second block. There data corroborate the successful synthesis of Z-

Lys-DIPS diblock copolypeptides. Moreover they suggest that homopolypeptides of DIPS described above were synthesized as well, although they did not possess the typical polypeptide spectral signatures.

2.5 Synthesis of Phosphonate NCAs

The data gathered during the synthetic studies of DMPS, DEPS, and DIPS suggest that isopropyl groups impart sufficient solubility to the phosphoserine NCA to yield soluble homopolymers and diblock copolymers. DIPS served as a valuable research tool in selecting an appropriate protecting group, but could be used in the synthesis of poly-(phosphoserine) due to the conditions used in isopropyl group deprotections. With the exception of methyl groups, alkyl groups are generally only used in the protection of phosphonate moieties (Figure 2.15A). Attempting to deprotect poly-DIPS under these conditions would yield poly-serine, which would hardly be the most efficient route toward poly(serine) (Figure 2.15B).

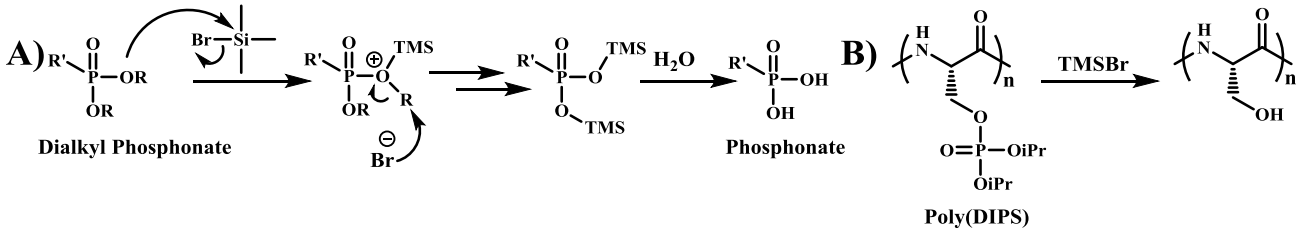


Figure 2.15. (A) Mechanism for deprotection of alkyl phosphonate. (B) Expected outcome from deprotection of poly(diisopropylphosphoserine) via TMSBr.[81]

Armed with knowledge gained from the work described above, diisopropylphosphonoalanine NCA (Pal NCA) was synthesized as a biomimetic analog of phosphoserine. Although the side chain of Pal is one atom shorter than serine, we theorized that the changes in properties would be minimal. In addition, Pal NCA could be synthesized starting from inexpensive and readily available amino acid derivatives. The synthesis started with preparation of the iodide **14**, which

was derived from the commercially available Z-Ser-OBzl. **14** was then converted to the protected diisopropylphosphonate **15** via an Arbuzov reaction[82] with triisopropylphosphite. It is noteworthy that the reaction was successful at 100 ^{o}C, which is lower than expected for an unactivated iodide. The carboxylate and the amine groups were then deprotected via

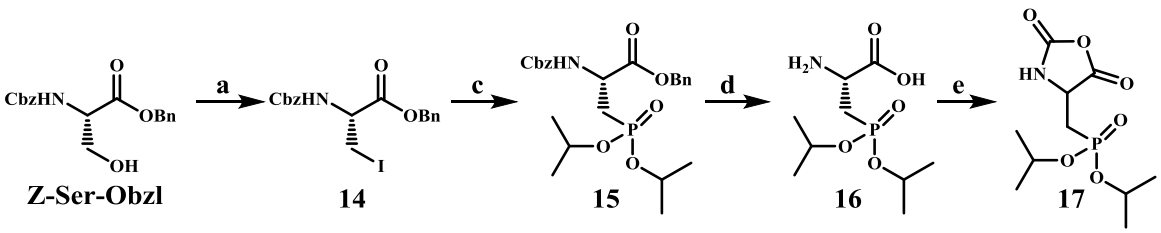


Figure 2.16. Synthesis of Pal NCA. (a) PPh_3, I_2, imidazole, CH_2Cl_2 (90% yield); (b) $P(OiPr)_3$, 100°C (85% yield); (c) H_2, Pd/C, MeOH (77% yield); (e) $COCl_2$, THF, 40°C (80% yield).

hydrogenolysis. NCA synthesis was achieved using phosgene in dry THF. The monomer was chromatographed in the glove box and recrystallized from THF/hexanes. Optical rotations of the precursor **16** and product **17** showed that racemization was occurring during phosgenation, which typically proceeds with no loss of optical purity.[83] A possible explanation is that at elevated temperatures during the synthesis of **17** fast, reversible elimination of diisopropylphosphite occurs, creating a dehydroalanine intermediate which racemizes the stereocenter (Figure 2.17).[84]

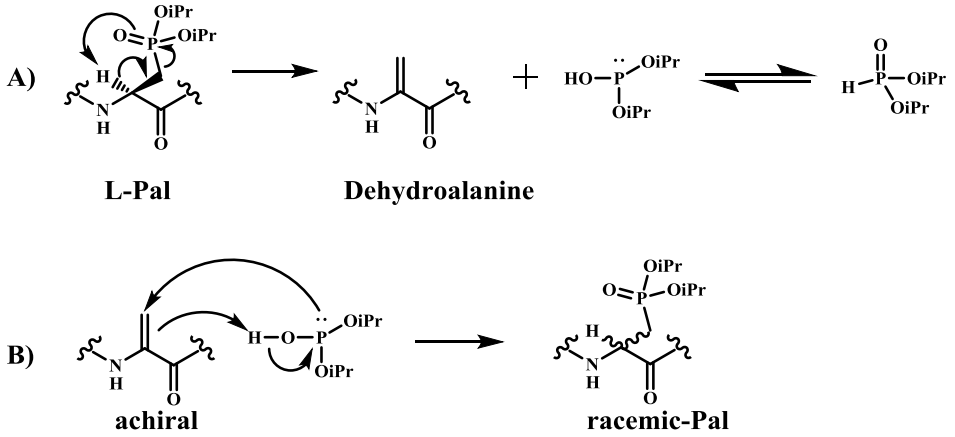


Figure 2.17. Possible reaction paths to explain observed racemization of L-Pal during NCA synthesis. (A) Facile elimination of diisopropylphosphite to yield dehydroalanine. (B) Fast addition of the diisopropylphosphite to regenerate Pal with loss of optical purity.

In addition to Pal NCA, Pha NCA, a 1-carbon homolog of Pal was also synthesized (Figure 2.1). The reasons for its synthesis were two-fold: to synthesize an isosteric analog to phosphoserine and to explore whether racemization could be avoided. Pha NCA was prepared starting with the commercially available l-homoserine, which was elaborated to the NCA via the same route that was used for Pal NCA.

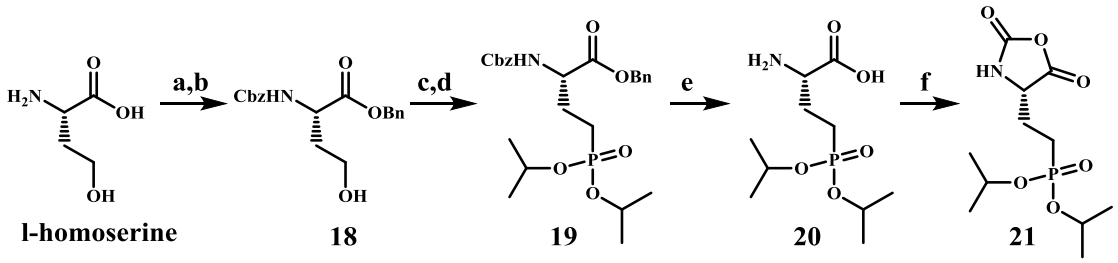


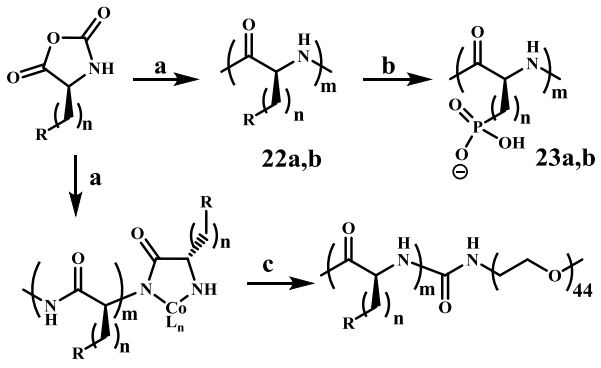
Figure 2.18. Synthesis of Pha NCA. (a) CbzCl, β-cylcodextrin, K_2CO_3, H_2O (89% yield);[85] (b) BnBr, Et_3N, acetone (85% yield); (c) PPh_3, I_2, imidazole, CH_2Cl_2 (88% yield); (b) $P(OiPr)_3$, 100°C (65% yield); (c) H_2, Pd/C, MeOH (88% yield); (e) $COCl_2$, THF, 40°C (68% yield).

Following chromatography and crystallization, the NCA **21** was obtained as a white, crystalline solid. Unlike **17**, **21** displayed an optical rotation, leading us to believe that racemization did not occur with this analog.

2.6 Phosphonate-Containing Polypeptides

Polymerization of NCAs **17** and **21** using $Co(PMe_3)_4$ in THF proceeded rapidly at ambient temperature to give the corresponding homopolypeptides **22a** and **22b** with complete monomer conversions and no reactions at the side chain phosphonate groups.[86] Residual Co salts were readily removed by washing the polypeptides with water. To see if chain length could be controlled, both **17** And **21** were separately polymerized to complete conversions at different monomer to initiator ratios (M:I), and active chains were then end capped with isocyanate-terminated PEG (M_n = 2000 Da).[87] Compositional analysis of purified, end capped polypeptides

A



R= P(O)(OiPr)$_2$; (a) n=1; b n=2

B

Monomer[a]	M_n[b]	M_w/M_n[c]	DP[d]	yield (%)[e]
20 Pal NCA	12 000	n/a	51	98
30 Pal NCA	17 600	n/a	75	99
40 Pal NCA	24 000	n/a	102	98
50 Pal NCA	30 600	n/a	130	97
60 Pal NCA	38 300	n/a	163	95
10 Pha NCA	5 480	1.07	22	94
20 Pha NCA	8 960	1.28	36	93
40 Pha NCA	17 900	1.17	72	91
60 Pha NCA	28 100	1.04	113	98
80 Pha NCA	41 800	1.03	168	93

Figure 2.19. Synthesis of phosphonohomopolypeptides. (A) Scheme for the synthesis of PEG-end capped homopolypeptides and deprotected homopolypeptides. (a) $Co(PMe_3)_4$, THF (see table to the right for yields); (b) TMSBr, $CHCl_3$, 60°C (70-90% yield); (c) PEG-NCO, 2kDa. (B) Table of results for homopolypeptides synthesis. [a] Number of equivalents of monomer per $Co(PMe_3)_4$. [b] Molecular weight determined for PEG end-capped samples using ^{1}H NMR. [c] Polydispersity index determined by GPC/LS analysis. [d] DP = degree of polymerization. [e] Total isolated yield of purified polypeptide. Pal NCA = 17; Pha NCA = 21. n/a = not applicable.

by ^{1}H NMR gave number average chains that increased linearly with M:I stoichiometry. Chain length distributions of **22b** samples were obtained by GPC/LS analysis and polydispersity

indices (M_w/M_n) were found to be between 1.03 and 1.28, indicating well-defined polypeptides were formed (Figure 2.19B). Due to lack of a refractive index (RI) difference from solvent, **22a** gave no RI signal in GPC analysis, and polydispersity indices could not be determined for these samples. Both **22a** and **22b** were obtained in high yields with precisely controlled chain lengths to over 150 residues long. These high molecular weight phosphono polypeptides were obtained in reaction times of less than 12 hours, as compared to previous studies where much longer reaction times (36 hours) were necessary to obtain shorter chains (40 residues).[74] These chain lengths, e.g., 25 to 150 residues, also cover a desirable range for many polypeptide material applications.[39] Overall, these data show that NCAs **17** and **21**, similar to other NCAs,[86] are able to undergo living polymerization when initiated with $Co(PMe_3)_4$.

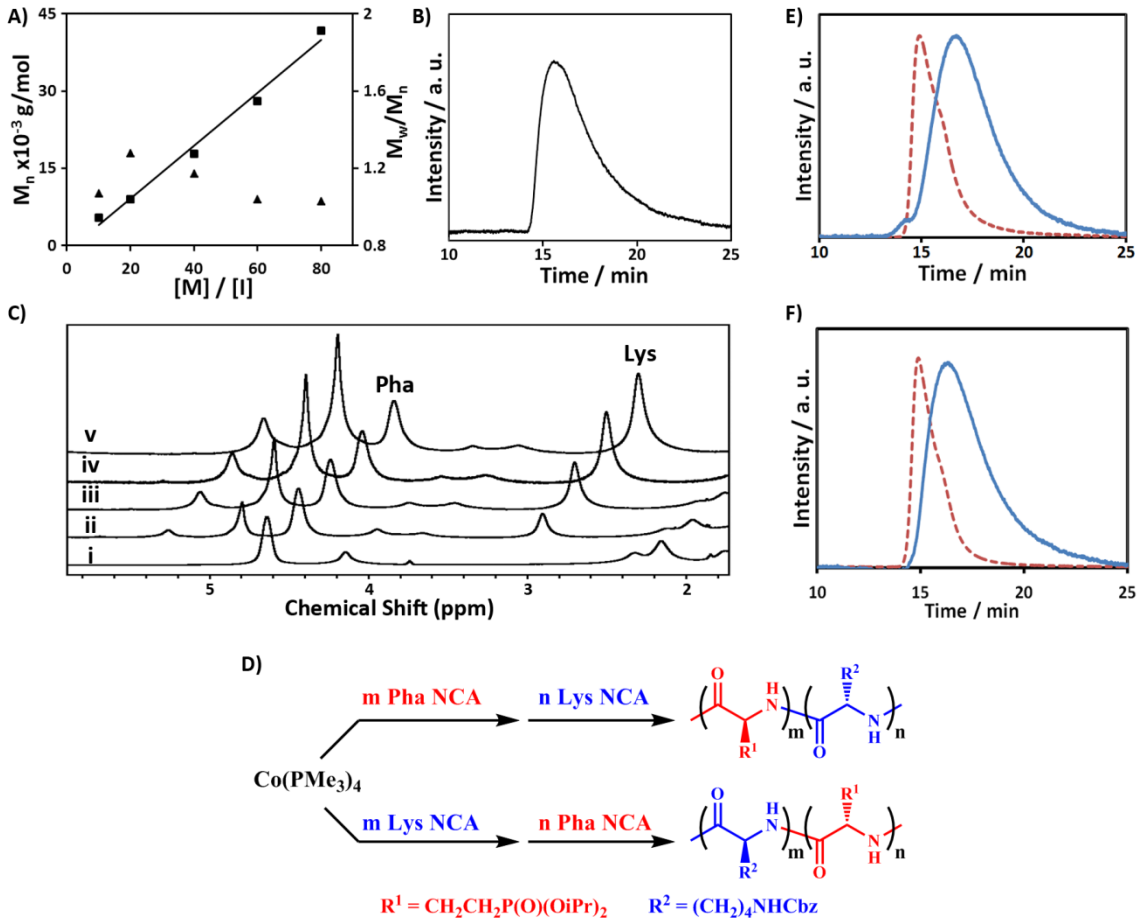


Figure 2.20. Synthesis of Pha homopolypeptides and diblocks. (A) Molecular weight (M_n, ▪) and polydispersity index (M_w/M_n, ▲) of poly(**5b**) as a function of monomer to initiator ([M]/[I]) ratio using $Co(PMe_3)_4$ in THF at ambient temperature. (B) GPC chromatogram (LS intensity in arbitrary units (a.u.) versus elution time) of a poly(**22b**) sample (Figure 2.19B, 40 Pha NCA). (C) ^{1}H NMR data showing different Lysine to Pha compositions obtained in block copolymerizations of Pha NCA and Lys NCA from Figure 2.19. Pha = POCH resonance of **22b** segment; Lys = NCH2 resonance of lysine segment; i = 40 Pha NCA; ii = 40 Pha NCA:20 Lys NCA; iii = 40 Pha NCA:40 Lys NCA; iv = 40 Pha NCA:60 Lys NCA; v = 40 Pha NCA:80 Lys NCA. (D) Schematic showing different block copolymer synthesis sequences. (E) GPC chromatogram of poly(**22a**)-block-Lys of 2 different degrees of polymerization (dotted line is figure 2.21 entry 5, solid line is entry 7). (F) GPC chromatogram of poly-Lys vs poly-Lys-block-(**22b**) of 2 different degrees of polymerization (solid line is poly Lys, M:I of 20:1; dotted line is figure 2.21 entry 10).

To further study the synthetic utility of these monomers, block copolypeptides were prepared using the phosphono NCAs **17** or **21** combined with Lys NCA as a model comonomer. All of these copolymerizations gave block copolypeptides in high yields and with low polydispersity indexes. The low polydispersity indexes of the block copolymers of Lys NCA and **17** also

provide indirect evidence that polymerization of **17** gives polypeptides with narrow chain length distributions. Block copolypeptides were prepared using different orders of monomer addition, allowing the synthesis of copolymers with a polyphosphonate segment on either the C- or N-terminal end. Such versatility is useful for incorporation of phosphono polypeptide domains at defined locations within block copolypeptide assemblies for controlled presentation of this functionality (Figures 2.20 and 2.21).

		First Segment[b]			Diblock Copolymer[c]			
First Monomer[a]	Second Monomer[a]	M_n	M_w/M_n	DP[d]	M_n	M_w/M_n	DP[d]	yield (%)[e]
20 Lys NCA	10 Pal NCA	12 600	1.12	48	19 600	1.02	78	97
20 Lys NCA	15 Pal NCA	12 600	1.12	48	22 400	1.07	90	94
20 Lys NCA	20 Pal NCA	12 600	1.12	48	24 300	1.01	98	97
20 Lys NCA	30 Pal NCA	12 600	1.12	48	27 400	1.05	111	90
20 Pal NCA	15 Lys NCA	12 000	n/a	51	25 600	1.13	100	90
20 Pal NCA	30 Lys NCA	12 000	n/a	51	33 100	1.13	151	92
20 Pal NCA	45 Lys NCA	12 000	n/a	51	55 100	1.05	209	90
20 Lys NCA	10 Pha NCA	14 900	1.12	57	21 400	1.14	83	94
20 Lys NCA	30 Pha NCA	14 900	1.12	57	36 100	1.10	142	91
20 Lys NCA	40 Pha NCA	14 900	1.12	57	43 800	1.06	173	94
40 Pha NCA	20 Lys NCA	17 900	1.15	72	24 300	1.07	96	94
40 Pha NCA	40 Lys NCA	17 900	1.15	72	32 300	1.05	127	99
40 Pha NCA	60 Lys NCA	17 900	1.15	72	39 900	1.06	156	95
40 Pha NCA	80 Lys NCA	17 900	1.15	72	42 800	1.19	167	98

Figure 2.21. Synthesis of phosphonate containing diblock copolypeptides. [a] Number of equivalents of monomer per $Co(PMe_3)_4$. [b] Molecular weight of first segments determined for PEG end-capped samples using ^{1}H NMR and polydispersity index determined by GPC/LS analysis where applicable. [C] Molecular weight of diblock copolymers determined for using ^{1}H NMR and polydispersity index determined by GPC/LS analysis. [d] DP = degree of polymerization. [e] Total isolated yield of purified diblock copolypeptide. n/a = not applicable.

Deprotection of homopolymers **22a** and **22b** was accomplished using TMSBr in chloroform at 60 ºC. Following dialysis against water and lyophilization, both poly(*rac*-phosphonoalanine) (**23a**), poly(Pal), and poly(*L*-phosphonohomoalanine) (**23b**), poly(Pha), were obtained in >90% yields. Both poly(Pal) and poly(Pha) were found to have good water solubility over a wide pH

range. The solution conformations of both polypeptides were examined by circular dichroism (CD) spectroscopy. As expected, poly(Pal) gave no CD signal since the stereocenters were racemized during monomer synthesis. Optically pure, poly(Pha) was found to give a CD spectrum consistent with a disordered chain conformation at pH 7.4 (Figure 2.22), similar to literature reports for poly(L-phosphoserine)[73] and the previously reported phosphonopolypeptides described above.[74] This result is not surprising since these polypeptides are highly charged polyelectrolytes at this pH. Since phosphonate groups have two acidic protons with pKa values of *ca.* 2.5 and 8.0, we also examined CD spectra of poly(Pha) samples) at pH values above and below these transitions. Although poly(Pha) with partially or fully deprotonated phosphonate groups were found to possess disordered conformations, as described above, the fully protonated, non-ionic poly(Pha) obtained at pH 1.0 was found to be predominantly α-helical, with characteristic minima at 208 and 222 nm (Figure 2.22).[88] Helical poly(Pha) was soluble in water and interconversion between α-helical and disordered conformations was readily accomplished by adjusting solution pH.

The pH responsive α-helical conformation of poly(Pha) is unprecedented for phosphate or phosphonate containing polypeptides, and may prove to be useful in subsequent efforts to direct self-assembly of block copolypeptides containing poly(Pha) segments. Since Pha is isosteric with *L*-phosphoserine, poly(Pha) and its copolymers may also prove useful in mimicking properties of phosphorylated proteins. The methods described here show that Pha and Pal based phosphono polypeptides can be prepared efficiently with controlled chain lengths, and are also readily incorporated into block copolymers with controlled architecture and composition.

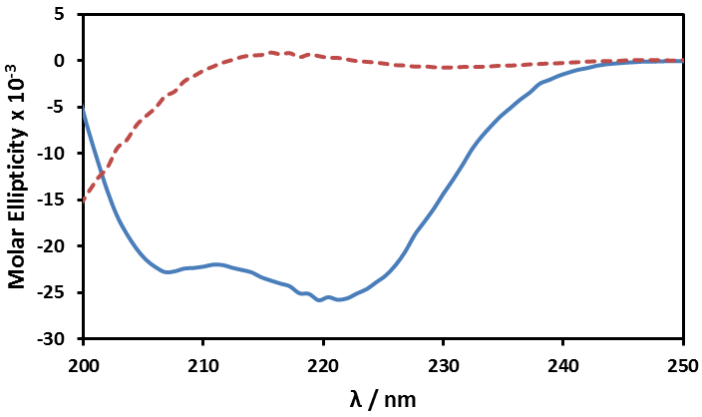


Figure 2.22. Circular dichroism spectra of poly(Pha)$_{36}$ (1 mg/ml) at 20 °C in water at pH 1.0 (0.1 M HCl, solid blue line) and pH 7.4 (10 mM PBS, dotted red line).

2.7 Conclusion

It has been shown that the isopropyl protecting group can impart sufficient solubility to phosphoserine-mimicking materials containing both phosphate and phosphonate functionality. The phosphonate-containing NCAs polymerize to form polypeptides of predictable molecular weight and composition. Deprotected homopolymers of poly(Pha) display a pH-responsive α-helix to random coil transition. These properties along with the polymers' scalable synthesis make them ideal candidates for bioengineering research. Collaborative efforts are under way to examine these polymers in biological settings.

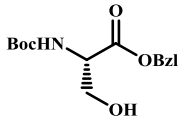
2.8 Experimental

2.8.1 Materials and Methods

Unless otherwise stated, reactions were carried out in oven-dried glassware under an atmosphere of nitrogen using anhydrous solvents. THF, hexanes, DCM, and toluene were purified by passage through activated alumina-packed columns under nitrogen. Pyridine was distilled from CaH_2 and was stored over 4Å molecular sieves in an airtight container. All reagents were ordered from Aldrich, Spectrum, Alfa Aesar, or Bachem, and were used as received unless otherwise stated. 1H NMR spectra were recorded at 500MHz on Bruker instruments; ^{13}C NMR spectra were recorded on 125MHz Bruker instruments with the solvent peak as internal reference. ^{31}P NMR spectra were recorded at 500MHz on a Bruker instrument and referenced using 85% aqueous, external H_3PO_4. Thin-layer chromatography (TLC) was conducted with EMD gel 60 F254 precoated plates (0.25 mm) and visualized using a combination of UV, permanganate, ninhydrin, and ceric ammonium molybdate staining. Selecto silica gel 60 (particle size 0.032–0.063 mm) was used for flash column chromatography. The silica was dried under vacuum at 200 ^{o}C for 2 days before it was used in the glove box. All Fourier Transform Infrared (FTIR) samples were prepared as thin films on NaCl plates and spectra were recorded on a Perkin Elmer RX1 FTIR spectrometer and are reported in terms of frequency of absorption (cm^{-1}). Tandem gel permeation chromatography/light scattering (GPC/LS) was performed on a SSI Accuflow Series III liquid chromatograph pump equipped with Wyatt DAWN EOS light scattering (LS) and Optilab rEX refractive index (RI) detectors. Separations were achieved using 10^5, 10^4, and 10^3 Å Phenomenex Phenogel 5 µm columns using 0.10 M LiBr in DMF as the eluent at 60 °C. GPC/LS samples were prepared at concentrations of 5 mg/mL, or higher if needed to increase signal to noise. Circular dichroism spectra were recorded on an OLIS RSM CD spectrophotometer

running in conventional scanning mode. Optical rotations were measured on a Rudolph Research Analytical Autopol III Automatic Polarimeter. NCA purifications and polymerization reactions were conducted in a nitrogen-filled glove box. Purified NCAs and cobalt initiator were stored in a -20 ^{o}C freezer inside of the glove box.

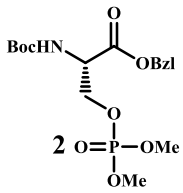
2.8.2 Experimental Procedures



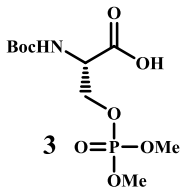
1

Benzyl (*tert*-bulylcarbonyl)-*L*-serinate (1). The following procedure is a modification of previously published work.[89] Boc-Ser-OH (10.0g, 48.7 mmol) were dissolved in 100ml of wet acetone. The flask was equipped with a condenser and a stir bar. Benzyl bromide (12.2 ml, 102 mmol) and triethylamine (12.3 ml, 87.7 mmol) were added to the stirred solution. The mixture was heated to 58^{o}C and was stirred for 2.5 hours. The absence of starting material was confirmed by TLC and the reaction was cooled to room temperature and solid byproducts were removed by filtration. The volatiles were then removed under reduced pressure and the resulting crude oil was dissolved in 100ml of ethyl acetate. The solution was then washed with 1.0M HCl (3x20ml) followed by brine (20ml). The organic layer was then dried over $MgSO_4$ and concentrated under reduced pressure. The resulting oil was suspended in 100ml of hexane and was heated to reflux. Once at reflux, ethyl acetate was slowly added until the mixture became homogeneous. The solution was allowed to reach room temperature and was then splaced into a 5^{o}C refrigerator. The crystallization was taken out of the fridge 1 hour later and the mother liquor was discarded. The white crystals were washed with hexane and were then dried under vacuum to yield the pure

product (13.1g, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (5H, br s), 5.49 (1H, br s), 5.21 (2H, br s), 4.43 (1H, s), 3.94 (2H, dd, *J*=35Hz, 5Hz), 2.35 (1H, br s), 1.44 (9H, s).

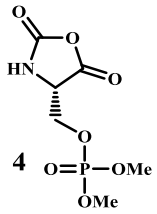


Benzyl *N*-(*tert*-butoxycarbonyl)-*O*-(dimethoxyphosphoryl)-*L*-serinate (2). 1 (3.08g, 10.4 mmol), carbon tetrabromide (6.92g, 20.8 mmol) and ~500mg of 4 Å molecular sieves were weighed into a 50ml roundbottom flask. The solids were dried under vacuum for 2 hours and were then dissolved in dry pyridine (15ml). The mixture was cooled to 0^{o}C using an ice bath and $P(OMe)_3$ (2.72 ml, 22.0 mmol) was added dropwise over 10 minutes. The reaction was stirred at 0^{o}C for 20 more minutes and was then allowed to warm up to room temperature. Once at room temperature, the solution was stirred for an additional 3 hours. The solution was then filtered and diluted with 100 ml of diethyl ether. The organic layer was washed with 1.0M HCl (25ml) and then with saturated $NaHCO_3$ (25 ml). The organic layer was then dried over $MgSO_4$, concentrated under reduced pressure, and chomatographed using 3/2 hexane/ethyl acetate → 1/1 hexane/ethyl acetate. The product was obtained as a clear, yellow oil, and was immediately used in the next step.



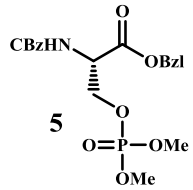
***N*-(*tert*-butoxycarbonyl)-*O*-(dimethoxyphosphoryl)-*L*-serine (3). 2** from the procedure above was dissolved in 15ml of wet methanol. The solution was stirred and 10% palladium on carbon

(380mg) was added, resulting in a black suspension. The suspension was sparged with hydrogen and was then stirred under 1atm of H_2 overnight. The next day the reaction was filtered through celite and the solvent was evaporated under reduced pressure, yielding the product as a clear, yellow oil (80 % over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.66 (1H, d, *J*=7.25), 4.50 (2H, br s), 4.30 (1H, br s), 3.80 (3H, d, *J*=1.4Hz), 3.78 (3H, *J*=1.4 Hz), 1.46 (9H, br s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 155.5, 80.4, 67.7, 54.8 (m), 53.7 (d, J_{CP}=7.0Hz),50.86 (MeOH), 28.2.

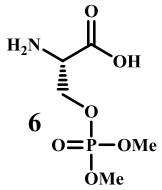


4

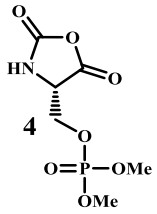
Synthesis of Dimethylphosphoserine NCA via Ghosez Reagent (DMPS) (4). 3 (245 mg, 0.782 mmol) was dried under high vacuum for 1 hour and was dissolved in 6 ml of dry THF. 1-chloro-*N*,*N*-dimethyl-2-propenylamine (206 µl, 1.56 mmol) were then added via a syringe and the reaction was stirred at room temperature under a nitrogen atmosphere for 48 hours. The solvent was then removed under high vacuum and the residue was pumped into the glove box. The oil was resuspended in 500 µl of THF and multiple crystallization conditions were attempted. Crystallization by dissolving the crude in 200 µl of ethyl acetate and layering this solution under 2ml of toluene in the freezer overnight gave a small amount of crystals suspended in oil. The crystals were dried and examined by NMR (<20% of partially crystallized oil). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (1H, s), 7.17 (toluene), 5.30 (DCM), 4.52 (1H, s), 4.42 (1H, dd, *J*=11.5Hz, 2.6Hz), 4.36 (1H, dd, *J*=11.2Hz, 3.0Hz), 3.79 (6H, dd, *J*=11.2Hz, 9.4Hz), 2.35 (toluene). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.2, 151.7, 125.3-129.0 (toluene), 65.0 (d, J_{CP}=5.2Hz), 58.3 (d, J_{CP}=5.9Hz), 55.0 (dd, J_{CP}=16.4Hz, 6.2Hz), 21.4 (toluene).



Benzyl *N*-((benzyloxy)carbonyl)-*O*-(dimethoxyphosphoryl)-*L*-serinate (5). Z-Ser-OBzl (4.80g, 14.6 mmol), carbon tetrabromide (9.65g, 29.1 mmol) and ~500mg of 4 Å molecular sieves were weighed into a 50ml roundbottom flask. The solids were dried under vacuum for 2 hours and were then dissolved in dry pyridine (15ml). The mixture was cooled to 0^oC using an ice bath and $P(OMe)_3$ (3.80 ml, 32.1 mmol) was added dropwise over 10 minutes. The reaction was stirred at 0^oC for 20 more minutes and was then allowed to warm up to room temperature. Once at room temperature, the solution was stirred for an additional 3 hours. The solution was then filtered and diluted with 100 ml of diethyl ether. The organic layer was washed with 1.0M HCl (25ml) and then with saturated $NaHCO_3$ (25 ml). The organic layer was then dried over $MgSO_4$, concentrated under reduced pressure, and chromatographed using 3:4 ethyl acetate:hexane. The solvent was removed under reduced pressure and the resulting solution was suspended in 20 ml of hexanes. The suspension was heated to 40^oC and ethyl acetate was added until it became homogeneous. The solution was then allowed to cool to room temperature and was then placed into a 5^oC refrigerator for 3 hours. The mother liqueur was then discarded and the solid was washed with hexane. The sample was then stripped of volatiles and a white solid with a light yellow tint was obtained (4.50g, 70-80%). NMR (500 MHz, $CDCl_3$) δ 7.36 (10H, br s), 5.83 (1H, d, *J*=8.0Hz), 5.22 (2H, s), 5.13 (2H, d, *J*=2.3Hz), 4.63 (1H, m), 4.48 (1H, m), 4.33 (1H, m), 3.66 (6H, dd, *J*=11.2Hz, 8.9Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.8, 155.8, 136.0, 134.9, 128.6, 128.6, 128.5, 128.4, 128.2, 128.1, 67.8, 67.2, 54.5.

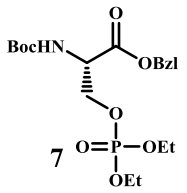


***O*-(dimethoxyphosphoryl)-*L*-serine (6). 5** (4.50g, 10.3 mmol) was dissolved in 30 ml of wet methanol. The solution was stirred and 10% palladium on carbon (450mg) was added, resulting in a black suspension. The suspension was sparged with hydrogen and was then stirred under 1atm of H_2 overnight. The next day the reaction was filtered through celite. 30ml of water was also ran through the filter as the product has poor solubility in methanol. The solution was concentrated under reduced pressure to about 10ml and the rest was removed by lyophilization to yield a yellowish white solid (100%). ^{1}H NMR (500 MHz, D_2O) δ 4.55 (1H, m), 4.48 (1H, m), 4.01 (1H, br s), 3.85 (3H, m), 3.83 (3H, m); ^{13}C NMR (125 MHz, D_2O) δ 170.2, 66.3 (d, J_{CP}=5.1Hz), 55.3 (dd, J_{CP}=6.1Hz, 2.6Hz), 54.3 (d, J_{CP}=8.8Hz).



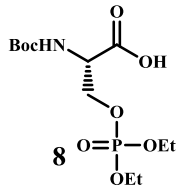
Synthesis of Dimethylphosphoserine NCA via phosgene (DMPS) (4) (2-85). 6 (929 mg, 4.36 mmol) was dissolved in 10ml of dry THF in a schlenk flask. Phosgene solution was then added via a syringe (4.3lml, 8.72 mmol, 20% in toluene). The reaction was stirred at 50^{o}C for 5 hours or until the solution was completely clear. The solvent was then removed under vacuum. **Note that phosgene is extremely toxic and an extended evacuation time of at least 4 hours is essential to remove all phosgene traces.** The phosgene in the vacuum traps was then quenched with 30% aqueous ammonia as did all of the syringes and glassware that made contact with the solution. The residue was then pumped into the glove box and was chromatographed using 60%

→ 100% THF/hexane as eluent to yield a clear, yellow oil (520mg, 50% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (1H, s), 4.52 (1H, s), 4.46 (1H, dd, *J*=11.5Hz, 2.6Hz), 4.36 (1H, dd, *J*=11.2Hz, 3.0Hz), 3.79 (6H, dd, *J*=11.1Hz, 9.1Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.2, 151.8, 65.0 (d, J_{CP}=5.2Hz), 58.3 (d, J_{CP}=5.9Hz), 55.0 (dd, J_{CP}=16.4Hz, 6.2Hz).

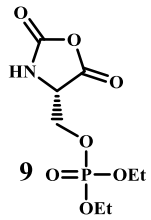


7

Benzyl *N*-(*tert*-butoxycarbonyl)-*O*-(diethoxyphosphoryl)-*L*-serinate (7). 1 (2.64 g, 8.94 mmol) and carbon tetrabromide (4.74 g, 14.3 mmol) were weighed into a 50ml roundbottom flask equipped with a stir bar. The solids were dried by purging with nitrogen for 30 minutes and were then dissolved in 8ml of dry pyridine. The resulting solution was cooled to 0^{o}C and $P(OEt)_3$ (2.76 ml, 16.1 mmol) was then added dropwise over a period of 10 minutes. Following the addition, the reaction was stirred at 0^{o}C for 1 hour. The solution was then warmed up to room temperature using a water bath and was stirred for 3 more hours. The reaction was then filtered and diluted to 100ml with diethyl ether. The organic phase was washed with 1.0M HCl (2x25ml) followed by saturated $NaHCO_3$ (25ml). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The yellow crude was chromatographed using 40 → 70% ethyl acetate/hexane. The solvent was removed under reduced pressure to yield the product as a clear, colorless oil (3.00g, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (5H, br s), 5.57 (1H, d, *J*=8.1Hz), 5.20 (2H, m), 4.55 (1H, m), 4.44 (1H, m), 4.29 (1H, m), 4.05 (4H, m), 2.03 (EtOAc), 1.44 (9H, s), 1.29 (3H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2, 155.2, 135, 128.6, 128.5, 128.3, 80.3, 67.6, 67.3 (d, J_{CP}=5.6Hz), 64.1 (t, J_{CP}=4.9Hz), 60.4, 54.1 (d, J_{CP}=7.3Hz), 28.3, 16.0 (d, J_{CP}=6.6Hz), 14.2.

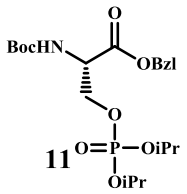


***N*-(*tert*-butoxycarbonyl)-*O*-(diethoxyphosphoryl)-*L*-serine (8). 7** (3.00 g, 6.95 mmol) were dissolved in 20 ml of wet ethanol and Pd/C (300mg, 10%) added to this solution. The suspension was sparged with hydrogen and was stirred under 1atm of hydrogen overnight. The reaction was then filtered through celite and concentrated under reduced pressure. The residue was dissolved in 30 ml of ethyl acetate and was extracted with 0.1 M NaOH (3x20ml). The water layers were combined and acidified with 1.0M HCl and was extracted with DCM (3x15ml). The DCM washes were combined, dried over $MgSO_4$, and stripped of volatiles under reduced pressure. The product was isolated as a clear, yellow oil (2.40 g, 90% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.67 (1H, d, *J*=7.4Hz), 4.49 (2H, m), 4.27 (1H, m), 4.13 (5H, m), 1.45 (9H, s), 1.33 (6H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 155.5, 80.3, 67.6 (d, J_{CP}=5.8Hz), 64.5-64.6 (3 overlapping peaks), 60.4, 53.7 (d, J_{CP}=7.6Hz), 28.3, 21.0, 16.0 (d, J_{CP}=6.6Hz), 14.2.



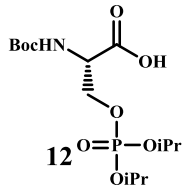
Diethylphosphoserine NCA (DIPS, 9). 8 (1.27g, 3.72 mmol) were dissolved in 10 ml of dry THF and Ghosez Reagent (734 µl, 5.55 mmol) was added via syringe. The solution was stirred under nitrogen overnight. The solvent was then removed under vacuum and the resulting orange oil was pumped into the box. The oil was chromatographed using 30 → 50% THF in hexane as eluent. Evaporation of the fractions containing the NCA yielded a clear, yellow oil (614 mg, 62% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (1H, s), 4.50 (2H, br m), 4.33 (1H, t, *J*=9.1Hz), 4.12

(4H, q, *J*=6.5Hz), 1.34 (6H, t, *J*=8.6Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.4, 151.7, 65.2 (d, J_{CP}=6.0Hz), 64.9, 58.4 (d, J_{CP}=6.3Hz), 15.9 (t, J_{CP}=6.9Hz).

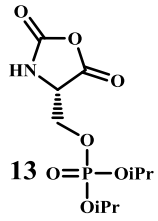


11

Benzyl *N*-(*tert*-butoxycarbonyl)-*O*-(diisopropoxyphosphoryl)-*L*-serinate (11) 1 (412 mg, 1.39mmol) and carbon tetrabromide (925 mg, 2.79mmol) were weighed into a septum vial equipped with a stir bar. The solids were dried by purging with nitrogen for 30 minutes and were then dissolved in 2ml of dry pyridine. The resulting solution was cooled to 0^{o}C and $P(OiPr)_3$ (757 µl, 3.07 mmol) was then added dropwise over a period of a few minutes. Following the addition, the reaction was stirred at 0^{o}C for 1 hour. The solution was then warmed up to room temperature using a water bath and was stirred for 2 more hours. The reaction did not contain any solids and was diluted to 30ml with diethyl ether. The organic phase was washed with 1.0M HCl (2x5ml) followed by saturated $NaHCO_3$ (5ml). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The yellow crude was chromatographed using 50/50 ethyl acetate/hexane. The solvent was removed under reduced pressure to yield the product as a clear, colorless oil (579 mg, 90% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (5H, br s), 5.60 (1H, d, *J*=6.2Hz), 5.19 (2H, d, *J*=1.4Hz), 4.57 (3H, m), 4.40 (1H, m), 4.26 (1H, m), 1.43 (9H, s), 1.29 (12H, m).



***N*-(*tert*-butoxycarbonyl)-*O*-(diisopropoxyphosphoryl)-*L*-serine (12) (2-280) 11** (579 mg, 1.31 mmol) was dissolved in 5ml of methanol. Pd/C (60mg, 10%) was then added to the solution and the mixture was sparged with hydrogen. The reaction was then allowed to stir under 1atm of hydrogen overnight. The next day the solution was filtered through celite and the volatiles were removed under reduced pressure yielding a clear oil, which solidified upon exposure to high vacuum (390 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (1H, s), 5.68 (1H, d, *J*=7.5Hz), 4.65 (2H, m), 4.48 (1H, m), 4.43 (1H, m), 4.23 (1H, m), 1.45 (9H, s), 1.32 (12H, d, *J*=6.0Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 155.5, 128.6, 80.1, 73.55 (d, J_{CP}=6.0Hz), 67.5, 67.4 (d, J_{CP}=6.1Hz), 53.7 (d, J_{CP}=8.4Hz), 28.3, 23.5 (m).

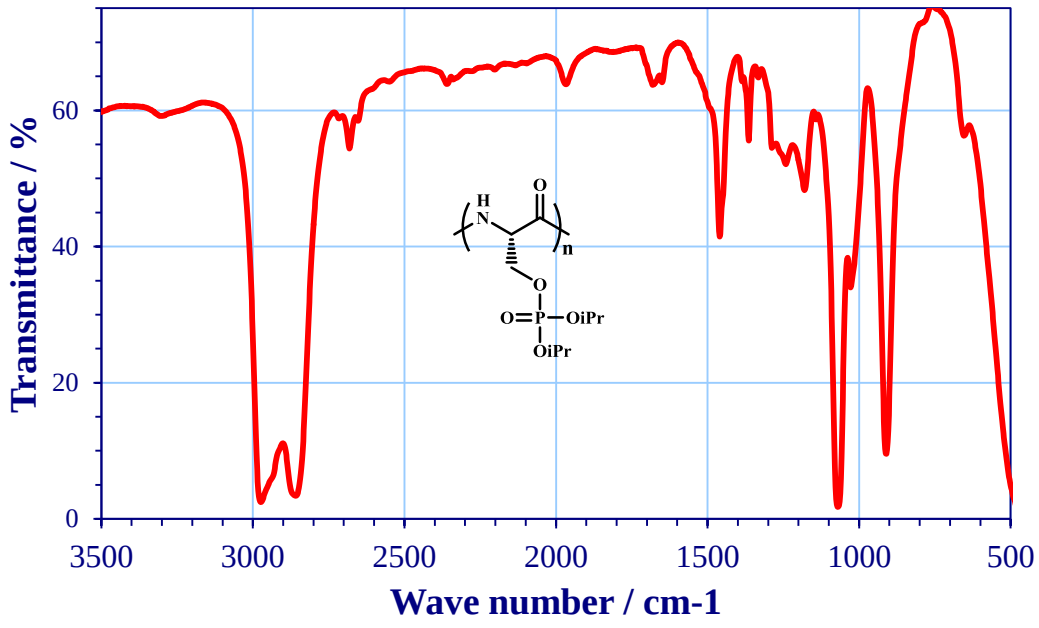


Diisopropylphosphoserine NCA (DIPS, 13) (3-7b). 12 (612 mg, 1.73 mmol) was dissolved in 5ml of dry THF. Ghozes Reagent (344 µl, 2.60 mmol) was then added via a syringe and the reaction was stirred at room temperature for 4 hours. The solvent was then removed under high vacuum and the resulting orange oil was brought into the glove box. The crude was then chromatographed using 30% THF in hexane. The resulting light yellow oil was dissolved in 1.7 ml of THF, was layered under 6 ml of hexane, and was left in the freezer overnight. The next day the mother liquor was discarded and the volatiles were removed under vacuum, yielding the product as a while solid (220 mg, 43%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (1H, br s), 4.63 (2H,

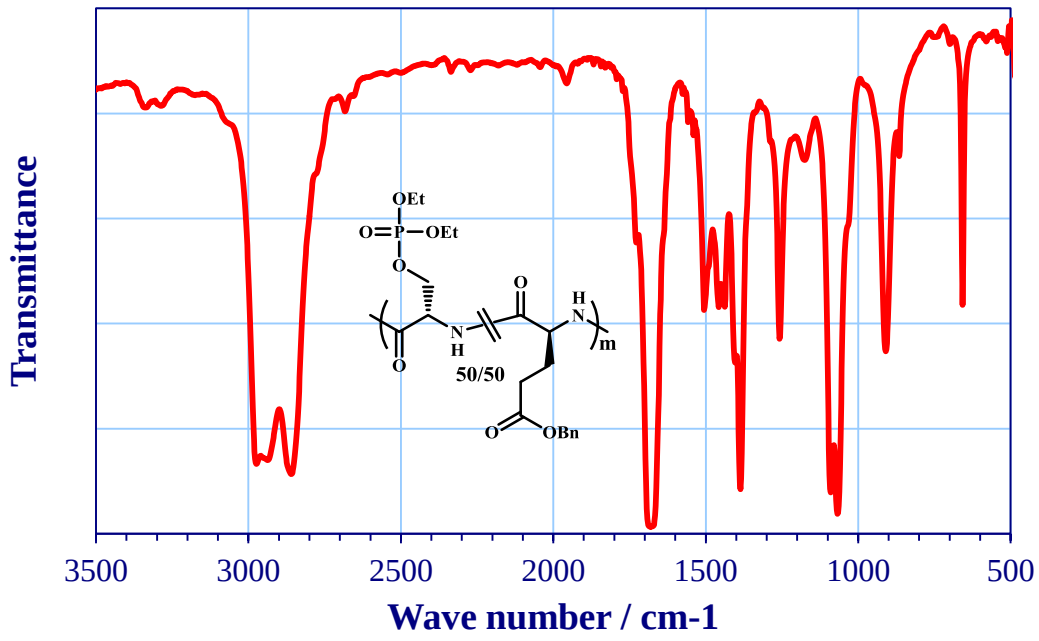
m), 4.54 (1H, br s), 4.29 (1H, t, J=7.4Hz), 1.33 (12H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.5, 151.5, 74.5, 74.1, 58.4 (d, J_{CP}=6.3Hz), 23.4 (m).

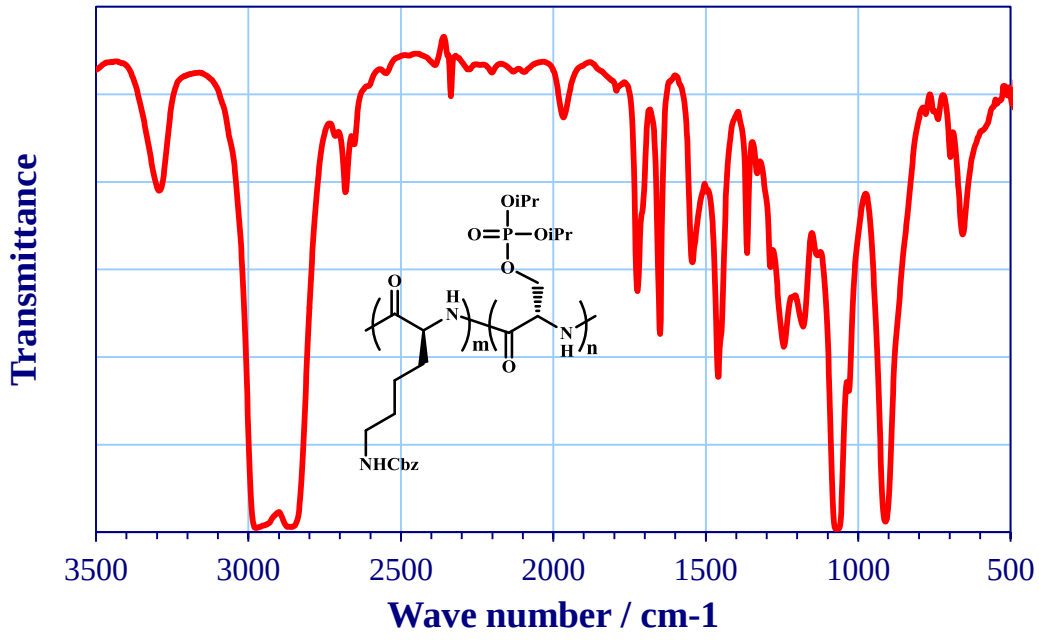
General procedure for polymerization of alkyl protected phosphoserine NCAs.

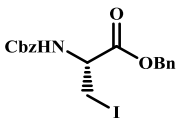
All polymerizations were performed in a nitrogen filled glove box using anhydrous, nitrogen purged solvents. To a solution of Pal NCA or Pha NCA in THF or DMF (50 mg/ml) was added a solution of $Co(PMe_3)_4$ in THF (10 mg/ml) to yield a desired monomer to initiator (M:I) ratio. The reactions were let stand and were allowed to react for 6 to 12 hours, depending on M:I ratio. M:I ratios of up to 40:1 were generally complete within 6 hours, higher ratios were let run for 16 hours. Reaction progress was monitored by FTIR by following the disappearance of the NCA band at 1792 cm^{-1}. The polypeptides were isolated by precipitation into water. The yields were not determined as the polypeptides were mainly synthesized for characterization purposes. Only IRs and GPCs were obtained, below is a representative IR of dialkylphosphoserine homopolymers. Note that these polymers display a stretch at ~1690 cm^{-1} instead of the usual 1650 cm^{-1} and 1540cm^{-1}.



General procedure for the synthesis of phosphonate containing diblock copolypeptides. All polymerizations were performed in a nitrogen filled glove box using anhydrous, nitrogen purged solvents. To a 50 mg/ml solution of the first NCA monomer in THF or DMF was added a solution of $Co(PMe_3)_4$ in THF (10 mg/ml) to yield a desired monomer to initiator (M:I) ratio. The reaction was monitored by FTIR and, upon complete consumption of the first monomer, a small aliquot was removed for molecular weight analysis via GPC-LS. To the remaining active polypeptide chains was added a solution of the desired quantity of second NCA monomer (50 mg/ml in THF or DMF). The reaction was allowed to stand overnight and was then checked for complete monomer consumption by FTIR. For random copolymers both of the NCAs were added at the same time. The yields were not determined as the polypeptides were mainly synthesized for characterization purposes. Below is a representative IR of copolymers.



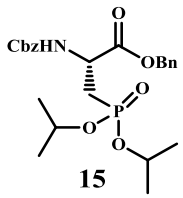




14

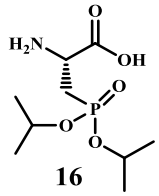
Benzyl (*R*)-2-(benzyloxycarbonylamino)-3-iodopropanoate. (14) The following procedure is a modification of previously published work.[89] Triphenylphosphine (3.1 g, 12 mmol) and imidazole (0.80 g, 12 mmol) were weighed into a 250 ml schlenk flask equipped with a stir bar. The solids were dried under vacuum for 20 minutes and the flask was back-filled with nitrogen gas. 100 ml of DCM were added via cannula and the resulting solution was cooled to 0 ^{o}C. Iodine (3.0 g, 12 mmol) was then added under a blanket of nitrogen, and the reaction was then warmed to room temperature for 10 minutes followed by cooling back to 0 ^{o}C. N-Benzyloxycarbonyl-L-serine benzyl ester (3.0 g, 9.1 mmol) was then added as a solution in 20 ml of dry DCM. The reaction was stirred at 0 ^{o}C for 2 additional hours at which point the starting material had disappeared by TLC (40% ethyl acetate in hexanes). Any solids that formed were then filtered off using a glass frit funnel and the resulting deep red solution was concentrated on

a rotary evaporator. The crude orange oil was then chromatographed using 40% ethyl acetate in hexanes as eluent. The volatiles were then removed on a rotary evaporator yielding a clear oil, which turned into a white solid upon drying (3.6 g, 8.8 mmol, 97 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.41 (10H, m, Ph), 5.65-5.68 (1H, d, *J*=7.1 Hz, NH), 5.20-5.24 (2H, d, *J*=3.1 Hz, CH_2Ph), 5.12-5.16 (2H, d, *J*=3.6 Hz, CH_2Ph), 4.60-4.65 (1H, dt, *J*=7.23 Hz, 7.23 Hz, CH), 3.56-3.64 (2H, m, CH_2I); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 155.4, 135.9, 134. 7, 128.7, 128.6, 128.6, 128.5, 128.3, 128.1, 68.1, 67.3, 54.0, 7.3.



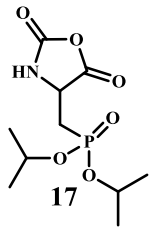
15

Benzyl (*R*)-2-(benzyloxycarbonylamino)-3-(diisopropoxyphosphoryl)propanoate (15). Benzyl (*R*)-2-(benzyloxycarbonylamino)-3-iodopropanoate (7.3 g, 17 mmol) was dried under vacuum for 20 minutes in a 50 ml round-bottom flask. Triisopropylphosphite (16 ml, 72 mmol) was then added via a syringe. The resulting suspension was stirred and heated to 100 ^{o}C, yielding a clear and colorless homogeneous mixture, which was then stirred under nitrogen overnight. The next morning the excess phosphite was removed via vacuum distillation at 80 ^{o}C. The resulting oil was chromatographed using 50% ethyl acetate in hexanes as eluent to remove any traces of residual phosphite. The solvent was removed to yield a clear, light yellow oil. 7.34 g (85 %, containing 30 mol. % of diisopropyl isopropylphosphonate). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28-7.37 (10H, m, Ph), 5.97-6.03 (1H, d, *J*=7.9 Hz, NH), 5.15-5.20 (2H, d, *J*=3.5 Hz, CH_2Ph), 5.08-5.14 (2H, m, CH_2Ph), 4.54-5.73 (3.7H, m, methine H from product and impurity), 2.23-2.37 (2H, dt, *J*=17.4 Hz, 7.1 Hz, CH_2P), 1.23-1.34 (17H, m, CH_3 from product and impurity). ^{31}P NMR (300 MHz, $CDCl_3$) δ 24 (product), -3 (impurity).



16

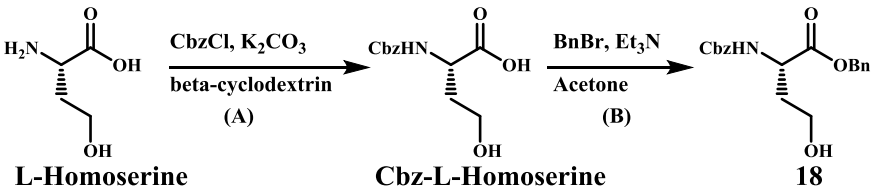
(R)-2-amino-3-(diisopropoxyphosphoryl)propanoic acid (16). Benzyl (*R*)-2-(benzyloxycarbonylamino)-3-(diisopropoxyphosphoryl)propanoate (7.2 g, containing 30 mol. % of diisopropyl isopropylphosphonate, *ca.* 13 mmol) was dissolved in 25 ml of wet MeOH. 800 mg of 5% Pd/C were added to the solution. The mixture was then sparged with hydrogen and stirred overnight under 1 atm of hydrogen. The reaction was then filtered through celite and concentrated on the rotary evaporator. The resulting yellow semisolid was dissolved in 50 ml of water and was extracted with 50 ml of ethyl acetate. The organic layer was washed with water (5 ml x 2). The combined aqueous phases were then concentrated on the rotary evaporator until the volume was roughly 10 ml and the remainder was lyophilized to yield a white solid (2.6 g, 10.4 mmol, 77 %). $[\alpha]_D^{25} = -8.2^o$ (c=10 mg/ml MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54-8.83 (2.6H, bs, NH_2/COOH), 4.62-4.75 (2H, m, OCH), 3.77-3.85 (1H, dd, *J*=12.6 Hz, CH), 2.53-2.63 (1H, t, *J*=17.2 Hz, C**H**HP), 2.31-2.42 (1H, q, *J*=13.3 Hz, CH**H**P), 1.27-1.36 (12H, m, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ171.4 (**C**OOH), 71.2-71.5 (dd, J_{CP}=21.6 Hz, 6.18Hz, O**C**H), 49.8 (**C**H), 26.9-28.1 (d, J_{CP}=141.3 Hz, **C**H_2P), 23.8-24.1 (m, **C**H_3); HRMS-ESI (*m/z*) [M + H] Calcd. for $C_9H_{21}NO_5P$, 254.1157; found 254.1170.



17

***rac*-Diisopropylphosphonoalanine N-carboxyanhydride, Pal NCA (17).** (R)-2-amino-3-(diisopropoxyphosphoryl)propanoic acid (490 mg, 1.9 mmol) was dried under vacuum for 30

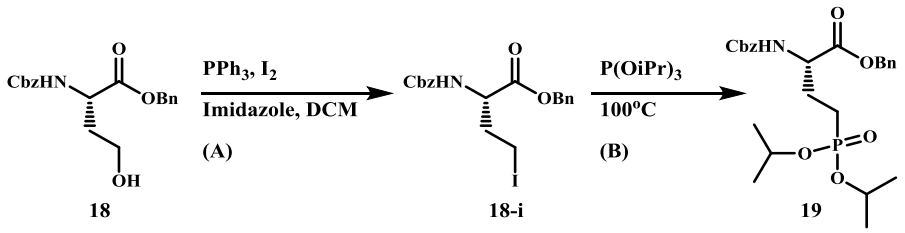
minutes in a 50 ml Schlenk flask. 10 ml of THF were added via a syringe followed by phosgene (1.9 ml of a 20 % solution in toluene, 3.9 mmol). The suspension was heated to 50 ^{o}C and was stirred under nitrogen for 2.5 hours. The resulting clear, pale yellow solution was stripped of solvent using the Schlenk line vacuum, and the resulting oil was transferred into a glove box under vacuum. The crude oil was then chromatographed in the glove box using 40 to 60 % THF in hexanes as eluent. The solvent was evaporated from the purified fraction, yielding a white solid. The solid was then recrystallized 3 times by dissolving in 2 ml of THF and layering under 10 ml of hexanes. The crystals were dried under vacuum after each recrystallization. The product was obtained as a white, crystalline solid (440 mg, 1.6 mmol, 82 %). $[\alpha]_D^{25} = 0.9^{o}$ (c = 10 mg/ml MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.83-6.90 (1H, s, NH), 4.70-4.80 (2H, m, OCH), 4.44-4.51 (1H, td, J=11.9Hz, 2.7Hz CH), 2.34-2.49 (1H, ddd, *J*=15.3Hz, 2.7Hz, 0.1Hz, C**H**HP), 1.97-2.06 (1H, m, CH**H**P), 1.26-1.38 (12H, m, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ168.8-169.0 (d, J_{CP}=21.1Hz, CH**C**OOCON), 150.6 (CHCOO**C**ON), 71.9-72.2 (dd, J_{CP}=38.6 Hz, O**C**H), 52.9-53.0 (d, J_{CP}=6.1Hz, **C**H), 29.0-30.2 (d, J_{CP}=145.56 Hz, **C**H_2P), 23.9-24.1 (m, **C**H_3). ^{31}P NMR (300 MHz, $CDCl_3$) δ 22. FTIR (THF): 1866, 1792 cm^{-1}; HRMS-ESI (*m/z*) [M-H] Calcd for $C_{10}H_{17}NO_6P$, 278.0794; found 278.0871.



Cbz-L-Homoserine. The following procedures have been modified from previously published works.[85,89] Homoserine (10 g, 84 mmol) and β-cyclodextrin (4.8 g, 4.2 mmol) were dissolved in 250 ml of 0.5 M K_2CO_3. Once a solution was obtained, benzyl chloroformate (13 ml, 92 mmol) was then added over the course of ~2 minutes. The reaction was then stirred vigorously until it

became homogenous. The solution was then transferred into a separatory funnel and was extracted with ethyl acetate (3 x 100 ml). The aqueous layer was then acidified to pH 1 with concentrated hydrochloric acid. The solution was then washed with ethyl acetate (4 x 100 ml), the organic washes were combined, dried over $MgSO_4$ and concentrated on the rotary evaporator to yield N-benzyloxycarbonyl-L-homoserine as a clear, colorless oil (19 g, 89 %) No further purification was necessary. The spectral properties were identical to those previously reported.[90]

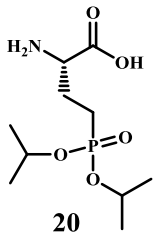
N-Benzyloxycarbonyl-L-homoserine benzyl ester. (18) N-Benzyloxycarbonyl-L-homoserine (19 g, 75 mmol) was dissolved in 200 ml of wet acetone. The solution was stirred and benzyl bromide (13 ml, 110 mmol) and triethylamine (15 ml, 110 mmol) were added to the mixture. The reaction was equipped with a condenser and was heated to 58 ^{o}C for 4 hours. Upon cooling to room temperature, a solid formed and was removed by filtration. The filtrate was concentrated on a rotary evaporated to yield a yellow oil with suspended solids. The oil was dissolved in 300 ml of ethyl acetate and the solution was transferred to a separatory funnel. The mixture was washed with 0.2 M HCl (2 x 75 ml) and once with 75 ml of brine. The organic layer was dried over $MgSO_4$ and concentrated on a rotary evaporator. The resulting crude oil was purified by silica chromatography using 20% to 80% ethyl acetate in hexanes as eluent. Removal of solvent yielded a clear, colorless oil, which was then mixed with 15 ml of benzene and lyophilized. The product was obtained as a white solid (22 g, 85 %), and the spectral properties were identical to those previously reported.[90]



Benzyl (S)-2-(benzyloxycarbonylamino)-4-iodobutanoate (18-i). The following procedure has been modified from previously published work.[89] Triphenylphosphine (5.0 g, 19 mmol) and imidazole (1.3 g, 19 mmol) were placed into a 250 ml Schlenk flask equipped with a stir bar and were dried under vacuum. 125 ml of dry DCM were then added via canula and the resulting solution was cooled to 0 ^{o}C. Iodine (4.8 g, 19 mmol) was then added under a blanket of nitrogen, the reaction was stirred and warmed to room temperature for 10 minutes followed by cooling back to 0 ^{o}C. N-benzyloxycarbonyl-L-homoserine benzyl ester (5.0 g, 15 mmol) was then added as a solution in 20 ml of dry DCM. The reaction was stirred at 0 ^{o}C for 2 more hours at which point the starting material had disappeared by TLC (30% ethyl acetate in hexanes). Any solids that formed were then filtered off using a glass frit funnel and the resulting deep red solution was concentrated on a rotary evaporator. The crude orange oil was then chromatographed using 20% to 30% ethyl acetate in hexanes as eluent. The volatiles were then removed on a rotary evaporator yielding a white solid (5.8 g, 13 mmol, 88 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30-7.40 (10H, br s, Ph), 5.31-5.36 (1H, d, *J*=7.6 Hz, NH), 5.16-5.22 (2H, s, CH_2Ph), 5.09-5.14 (2H, s, CH_2Ph), 4.44-4.49 (1H, m, CH), 3.08-3.17 (2H, m, CH_2I), 2.40-2.50 (1H, m, C**H**HCH_2I), 2.17-2.27 (1H, m, CH**H**CH_2I); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 155.95, 134.05, 134.9, 128.8, 128.7, 128.7, 128.6, 128.4, 128.3, 128.2, 67.69, 67.2, 54.8, 36.9.

Benzyl (S)-2-(benzyloxycarbonylamino)-4-(diisopropoxyphosphoryl)butanoate (19). Benzyl (S)-2-(benzyloxycarbonylamino)-4-iodobutanoate (5.8 g, 13 mmol) was dried under vacuum for 20 minutes in a 50 ml round-bottom flask. Triisopropylphosphite (14 ml, 61 mmol) was then

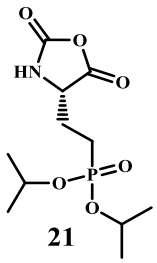
added via a syringe. The resulting suspension was stirred and heated to 100 ^{o}C, yielding a clear and colorless homogeneous mixture, which was then stirred under nitrogen overnight. The excess phosphite was then removed via vacuum distillation at 80 ^{o}C. The resulting oil was chromatographed using 70% ethyl acetate in hexanes as eluent to yield the product as a clear, yellow oil (4.2 g, 65 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30-7.40 (10H, br s, Ph), 5.54-5.58 (1H, d, *J*=7.85 Hz), 5.16-5.19 (2H, s, CH_2Ph), 5.09-5.11 (2H, s, CH_2Ph), 4.60-4.67 (2H, m, OCH), 4.43-4.48 (1H, m, CH), 2.11-2.21 (1H, m, C**H**HP), 1.90-2.00 (1H, m, CH**H**P), 1.61-1.77 (2H, m, CH_2), 1.23-1.30 (12H, dd, *J*=13.5 Hz, 6.2Hz, CH_3); HRMS-ESI (*m/z*) [M + H] Calcd for $C_{25}H_{34}NO_7P$, 492.2151; found 492.2144.



20

(S)-2-Amino-4-(diisopropoxyphosphoryl)butanoic acid (20). Benzyl (S)-2-(benzyloxycarbonylamino)-4-(diisopropoxyphosphoryl)butanoate (4.1 g, 8.4 mmol) was dissolved in 25 ml of wet MeOH. 600 mg of 5% Pd/C were added to the solution. The mixture was then sparged with hydrogen and stirred for 2 days under 1 atm of hydrogen. The next day the reaction was filtered through celite and was concentrated on the rotary evaporator. The resulting white solid was dissolved in 50 ml of water and was extracted with 50 ml of 80% ethyl acetate in hexanes. The combined aqueous layer was then concentrated on the rotary evaporator until the volume was roughly 10 ml and the remainder was lyophilized to yield a white solid (2.0 g, 88 %). $[\alpha]_D^{21}$ = +6.3 (c = 10 mg/ml MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05-8.63 (2.5H, br s, NH/COOH), 4.60-4.70 (2H, br s, OCH), 3.57-3.66 (1H, br s, CH), 2.10-2.24 (1H, br s, C**H**HP), 1.90-2.10 (3H, br s, CH**H**P and CH_2), 1.26-1.34 (12H, t, *J*=6.5 Hz); ^{13}C NMR

(125MHz, $CDCl_3$) δ 173.8, 70.2-70.5 (t, J_{CP}=7.6 Hz), 54.9-55.2(d, J_{CP}=17.2 Hz), 24.4, 24.0-24.1 (q, J_{CP}=3.9 Hz), 22.5-23.7 (d, J_{CP}=144 Hz); HRMS-ESI (*m/z*) [M + H] Calcd for $C_{10}H_{23}NO_5P$, 268.1314; found 268.1310.



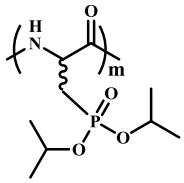
21

(S)-Diisopropylphosphonohomoalanine N-carboxyanhydride, Pha NCA, (21). (S)-2-Amino-4-(diisopropoxyphosphoryl)butanoic acid (880 mg, 3.3 mmol) was dried under vacuum for 30 minutes in a 50 ml Schlenk flask. THF (10 ml) was then added via a syringe followed by phosgene (3.3 ml of a 20% solution in toluene, 6.6 mmol). The reaction mixture initially clarified but then some solids separated shortly after. The suspension was heated to 40 ^oC and was stirred under nitrogen for 2.5 hours or until no more solids were observed. The resulting clear, pale yellow solution was stripped of solvent using the Schlenk line vacuum and the resulting oil was moved into a glove box under vacuum. The crude oil was then dissolved in 50% THF in hexanes, and was chromatographed in the glove box using 40 to 60% THF in hexanes as eluent. The fractions were analyzed for the presence of NCA using FTIR. The desired fractions were combined and solvent removed, yielding a white solid. The solid was then recrystallized 3 times by dissolving the residue in 1.5 ml of THF and layering under 7.5 ml of hexanes. The crystals were dried under vacuum after each recrystallization. The product was obtained as a white, crystalline solid (660 mg, 68 %). $[\alpha]_D^{24}$ = +3.7 (c = 10 mg/ml MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ 8.21-8.24 (1H, s, NH), 4.64-4.75 (2H, m, OCH), 4.32-4.36 (1H, dd, *J*=7.7Hz, 2.2Hz), 2.20-2.32 (1H, m, C**H**HP), 1.88-1.2.05(2H, m, CH**H**P and C**H**HC), 1.76-1.86

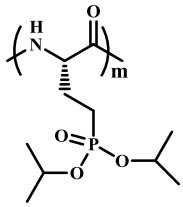
(1H, m, CH**H**C), 1.28-1.38 (12H, dt, *J*=6.4Hz, 2.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9, 151.3, 71.3-71.4 (t, J_{CP}=7.9 Hz), 57.7-57.8 (d, J_{CP}=10.5 Hz), 25.7-25.8 (d, J_{CP}=4.9 Hz), 23.9-24.0 (m), 22.7-23.9 (d, J_{CP}=145.0 Hz); ^{31}P NMR (500 MHz, $CDCl_3$) δ 28; FTIR (THF): 1864, 1792 cm^{-1}; HRMS-ESI (*m/z*) [M - H] Calcd for $C_{11}H_{19}NO_6P$, 292.0950; found 292.1010.

General procedure for polymerization of phosphonate containing NCAs.

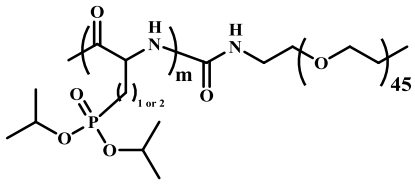
All polymerizations were performed in a nitrogen filled glove box using anhydrous, nitrogen purged solvents. To a solution of Pal NCA or Pha NCA in THF (50 mg/ml) was added a solution of $Co(PMe_3)_4$ in THF (10 mg/ml) to yield a desired monomer to initiator (M:I) ratio. The reactions were let stand and were allowed to react for 6 to 12 hours, depending on M:I ratio. M:I ratios of up to 40:1 were generally complete within 6 hours, higher ratios were let run for 16 hours. Reaction progress was monitored by FTIR by following the disappearance of the NCA band at 1792 cm^{-1}. The polypeptides were isolated by precipitation into water. Polypeptide was then collected by centrifugation and the solvents removed under vacuum to yield the products as white solids (yields 91-98 %).



Poly(*rac*-diisopropylphosphonoalanine) poly(22a). $[\alpha]_D^{25} = 1.0^o$ (c = 10 mg/ml MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.9-8.5 (1H, br d, NH), 4.4-4.9 (3H, br s, OCH and CH), 1.9-2.6 (2H, br m, CH_2P), 1.1-1.5 (12H, br s, CH_3). ^{31}P NMR (500 MHz, $CDCl_3$) δ 26. FTIR (THF): 1688 cm^{-1}.



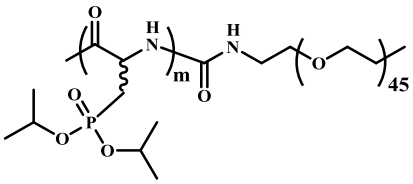
Poly(S-diisopropylphosphonohomoalanine), poly(22b). $[\alpha]_D^{21} = -3.3^o$ (c = 10 mg/ml MeOH). ^{1}H NMR (500MHz, $CDCl_3$) δ 7.99-8.20 (0.7H, br s), 4.51-4.74 (1.8H, br s), 4.05-4.25 (0.8H, br s), 1.66-2.43 (4H, multiple aliphatic signals), 1.16-1.44 (12H, br s). ^{31}P NMR (500 MHz, $CDCl_3$) δ 29. FTIR (THF): 1656, 1550 cm^{-1}.



General procedure for polypeptide endcapping with poly(ethylene glycol) and chain length determination by end group analysis.

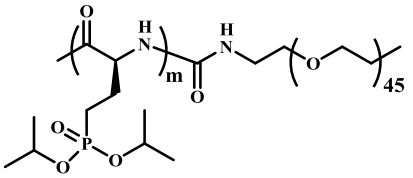
The general procedure for polymerization of Pal NCA (**22a**) and Pha NCA (**22b**) was followed. Upon completion of the reaction, as confirmed by FTIR, a solution of α-methoxy-ω-isocyanoethyl-poly(ethylene glycol), PEG-NCO (MW = 2000 Da, 3 equiv per $(PMe_3)_4Co$) in THF, was added to the polymerization reaction in a dinitrogen filled glove box. The reaction was stirred for 3 hours and then stripped of solvent under vacuum, and the resulting polymer film was suspended in 400 µl of deionized water. Use of a sonication bath was necessary to completely disperse the film. The polymer was then collected by centrifugation. Resuspension and centrifugation was repeated 2 additional times, and then water was removed from the final pellet by lyophilization. To determine polypeptide molecular weights (M_n), ^{1}H NMR spectra were obtained in deuterated chloroform. Since it has been shown that end-capping is quantitative for $(PMe_3)_4Co$ initiated NCA polymerizations when excess isocyanate is used,[87] integrations of the

methyl group resonances versus the polyethylene glycol resonance at δ 3.64 could be used to obtain polypeptide lengths (see examples below and in spectral data section).



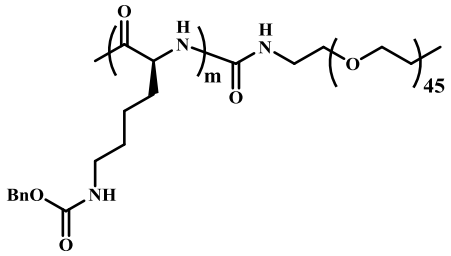
Spectral data for poly(*rac*-diisopropylphosphonoalanine)-*block*-PEG (20:1 M:I ratio, the PEG resonance at δ 3.6 was set to an integration value of 180H and compared to the aliphatic resonance at δ 1.0-1.5 for chain length determination).

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.5-4.8 (126H, br s, OCH and CH), 3.6-3.7 (180H, s, PEG), 2.0-2.6 (107H, br m, CH_2P), 1.0-1.5 (611H, br s, CH_3).



Spectral data for poly(S-diisopropylphosphonohomoalanine)-*block*-PEG (20:1 M:I ratio, the PEG resonance at δ 3.6 was set to an integration value of 180H and compared to the aliphatic resonance at δ 1.0-1.5 for chain length determination).

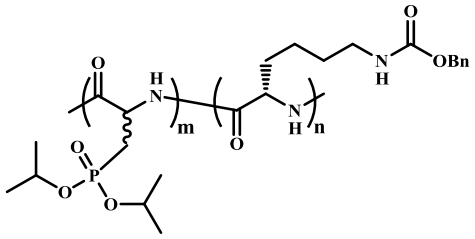
^{1}H NMR (500MHz, $CDCl_3$) δ 8.04-8.22 (16H, br s), 4.52-4.77 (65H, br s), 4.03-4.23 (24H, br s), 3.57-3.71 (180H, br s), 1.62-2.44 (151H, multiple aliphatic signals), 1.09-1.50 (433H, br s).



Spectral data for poly(Cbz-L-lysine)-*block*-PEG (20:1 M:I ratio, the PEG resonance at δ 3.6 was set to an integration value of 180H and compared to the CH_2N resonance at δ 3.0-3.2 for chain length determination).

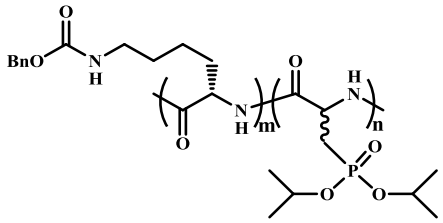
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17-7.27 (br s, overlap with $CDCl_3$), 5.42-5.52 (35H, br s), 4.93-5.10 (99H, br s), 3.77-3.94 (30H, br s), 3.57-3.69 (180H, br s), 2.99-3.19 (95H, br s), 1.81-2.04 (84H, br s), 1.14-1.69 (442H, overlapped aliphatic region).

General procedure for the synthesis of phosphonate containing diblock copolypeptides. All polymerizations were performed in a nitrogen filled glove box using anhydrous, nitrogen purged solvents. To a 50 mg/ml solution of the first NCA monomer in THF was added a solution of $Co(PMe_3)_4$ in THF (10 mg/ml) to yield a desired monomer to initiator (M:I) ratio. The reaction was monitored by FTIR and, upon complete consumption of the first monomer, a small aliquot was removed for molecular weight analysis via PEG end-capping or GPC-LS. To the remaining active polypeptide chains was added a solution of the desired quantity of second NCA monomer (50 mg/ml in THF). The reaction was allowed to stand overnight and was then checked for complete monomer consumption by FTIR. A small aliquot was removed for GPC analysis and the polypeptide was isolated by precipitation of the remainder into hexanes (2 ml per ~10 mg of polymer). The polymer was collected by centrifugation and was dried under vacuum to yield a white, stringy solid (91 to 98 %).



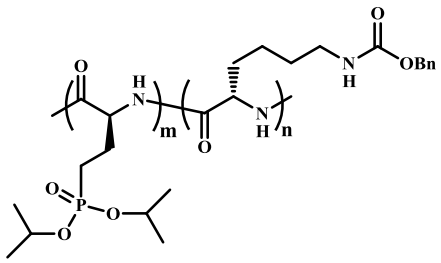
Spectral data for poly(*rac*-diisopropylphosphonoalanine)-*block*-poly(CBz-L-Lysine) (20:15:1 Pal:Lys:I ratio, the Pal OCH and CH resonances at δ 4.5-4.8 were set to an integration value of 126H, determined from a separate PEG end-capping experiment, and compared to the lysine CH_2N resonance at δ 3.0-3.2 for chain length determination).

^{1}H NMR (500 MHz, $CDCl_3$) δ7.91-8.50 (65H, br s), 7.16-7.37 (328H, s), 5.37-5.55 (33H, br s), 4.94-5.10 (91H, br s), 4.51-4.83 (126H, br s), 3.77-3.96 (30H, br s), 2.98-3.23 (94H, br s), 1.02-2.65 (1110H, overlapped aliphatic region).



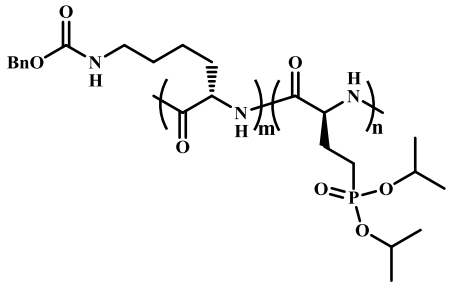
Spectral data for poly(CBz-L-Lysine)-*block*-poly(*rac*-diisopropylphosphonoalanine) (20:10:1 Lys:Pal:I ratio, the lysine CH_2N resonance at δ 3.0-3.2 was set to an integration value of 95H, determined from a separate PEG end-capping experiment, and compared to the Pal OCH and CH resonances at δ 4.5-4.8 for chain length determination).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.8-8.4 (48H, br s, NH of backbone), 7.0-7.5 (br s, Bn, overlaps with solvent peak), 5.4-5.5 (33H, br s, NH of lysine side chain), 4.9-5.1 (109H, br s, CH_2Ph), 4.5-4.8 (76H, br s, OCH and CH from Pal), 3.7-4.0 (30H, br s), 3.4-3.6 (12H, br s), 2.9-3.2 (95H, br s, NCH_2), 2.1-2.7 (26H, br s), 1.7-2.0 (82H, br s), 0.9-1.7 (796H, br s).



Spectral data for poly(S-diisopropylphosphonohomoalanine)-*block*-poly(CBz-L-Lysine) (40:20:1 Pha:Lys:I ratio, the Pha OCH resonance at δ 4.5-4.8 was set to an integration value of 124H, determined from a separate PEG end-capping experiment, and compared to the lysine CH_2N resonance at δ 3.0-3.2 for chain length determination).

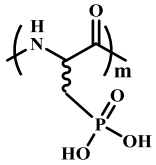
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83-8.52 (35H, br s), 7.10-7.35 (576H, br s), 4.88-5.13 (60H, br s), 4.52-4.74 (124H, br s), 2.92-3.26 (48H, br s), 0.93-2.54 (1521H, overlapping aliphatic signals).



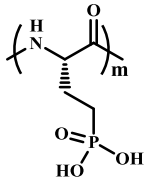
Spectral data for poly(CBz-L-Lysine)-*block*-poly(S-diisopropylphosphonohomoalanine) (20:10:1 Lys:Pha:I ratio, the lysine CH_2N resonance at δ 3.0-3.2 was set to an integration value of 114H, determined from a separate PEG end-capping experiment, and compared to the Pha OCH resonance at δ 4.5-4.8 for chain length determination).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88-8.45 (38H, br s), 7.09-7.34 (375H, br s), 5.36-5.56 (37H, br s), 4.91-5.13 (113H, br s), 4.52-4.74 (47H, br s), 3.77-3.96 (49H, br s), 2.96-3.23 (114H, br s) 1.03-2.36 (835H, overlapping aliphatic peaks).

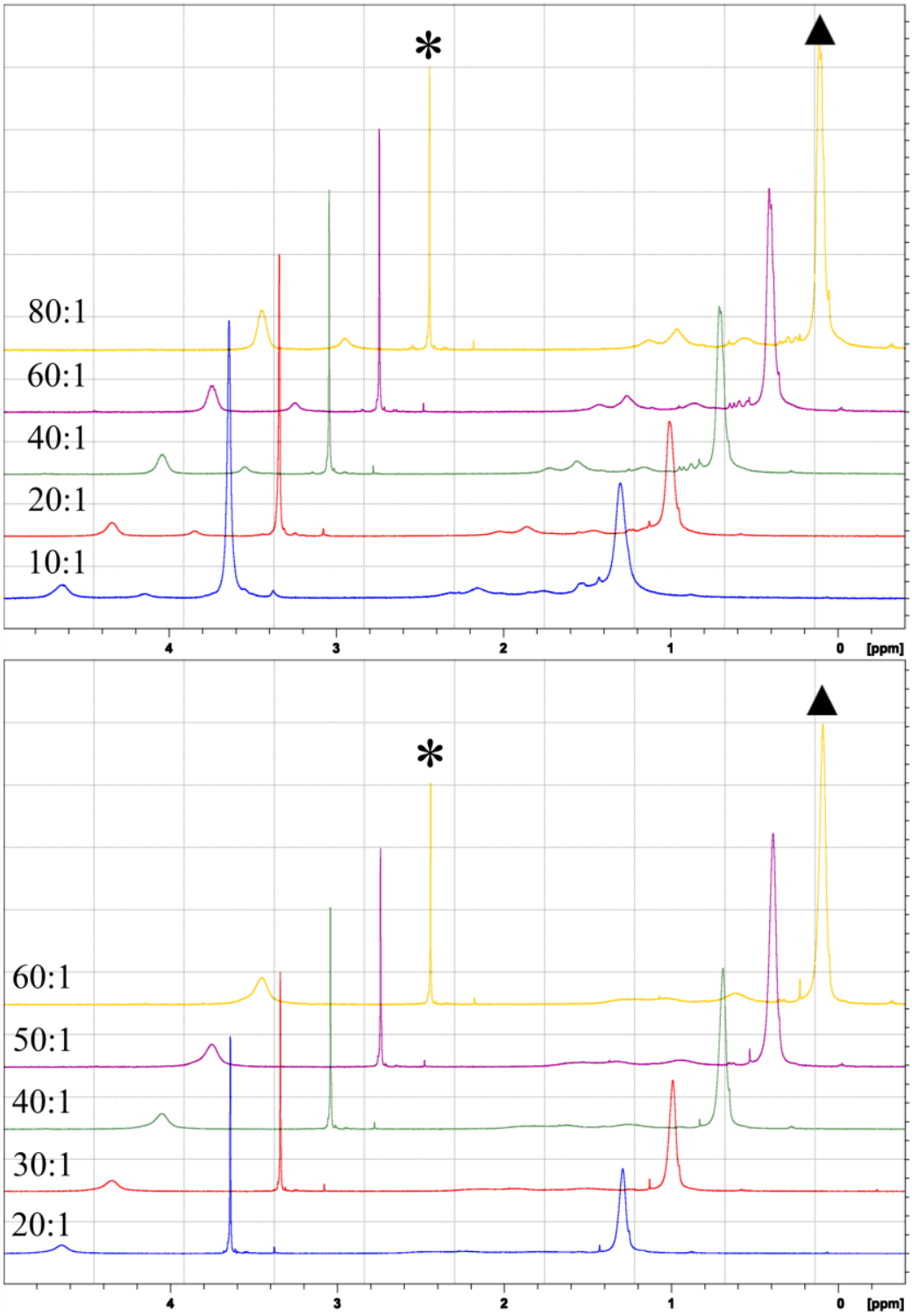
General procedure for the deprotection of phosphonate containing polypeptides. A sample of **22a** or **22b** (40 mg) was dissolved in 3 ml of dry chloroform in a 10 ml round-bottom flask equipped with a condenser. Bromotrimethylsilane (240 µl, 1.8 mmol) and triethylamine (200 µl, 1.4 mmol) were then added via syringe. The mixture was heated to 61 ^{o}C and stirred overnight under a nitrogen atmosphere. The next day the reaction was stripped of solvent under vacuum and the remaining residue was dissolved in 2 ml of deionized water. The resulting cloudy suspension was transferred to a 2000 MWCO dialysis membrane and was dialyzed against 4 L of water for 1 day, then 100 mM aqueous NaCl for 1 day, then water for 1 day, with twice daily dialyzate changes throughout. The sample was then removed from the dialysis bag and any particulates that remained were removed by filtration through a 45 µm syringe filter. The water was then removed by lyophilization to yield a fluffy, white solid (70 to 90 %).



Poly(*rac*-phosphonoalanine), 22a. ^{1}H NMR (500 MHz, D_2O) δ 4.37-4.65 (1H, br s), 1.99-2.28 (2H, br s); $[\alpha]_D^{25}$ = 0.1 (c = 10 mg/ml 0.1 M NaOH in H_2O).

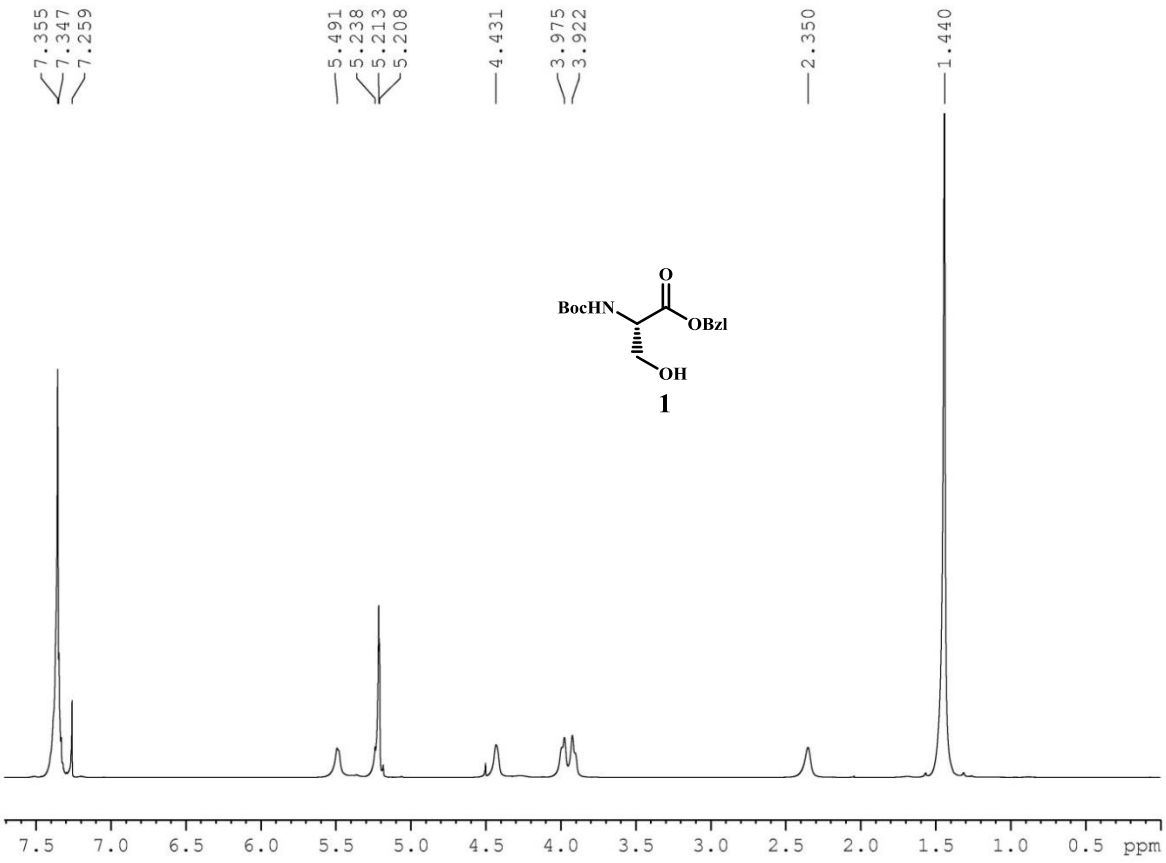


Poly(S-phosphonohomoalanine), 22b. ^{1}H NMR (500 MHz, D_2O) δ 4.34 (1H, br s), 3.90 (2H, br s), 2.07-1.96 (2H, br d); $[\alpha]_D^{25}$ = -61.8 (c = 10 mg/ml 0.1 M NaOH in H_2O).



^{1}H NMR spectra of PEG (2000 Da) end-capped A) poly(L-diisopropylphopshonohomoalanine), **22a**; and B) poly(rac-diisopropylphosphonoalanine), **22b**. The peak areas of the aliphatic polymer resonances increase relative to the constant PEG peak area as [M]/[I] ratio was increased.

2.8.2 NMR Data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5.213
5.208
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3.922
2.350
1.440
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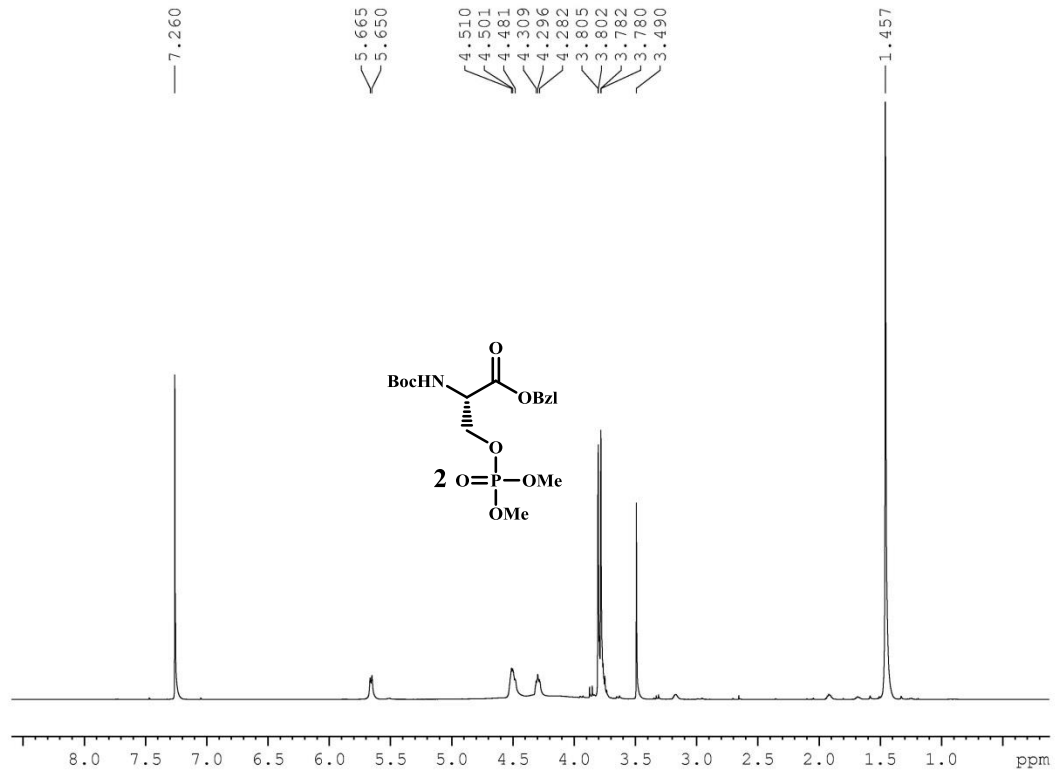
O
OBzl
OH
1
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm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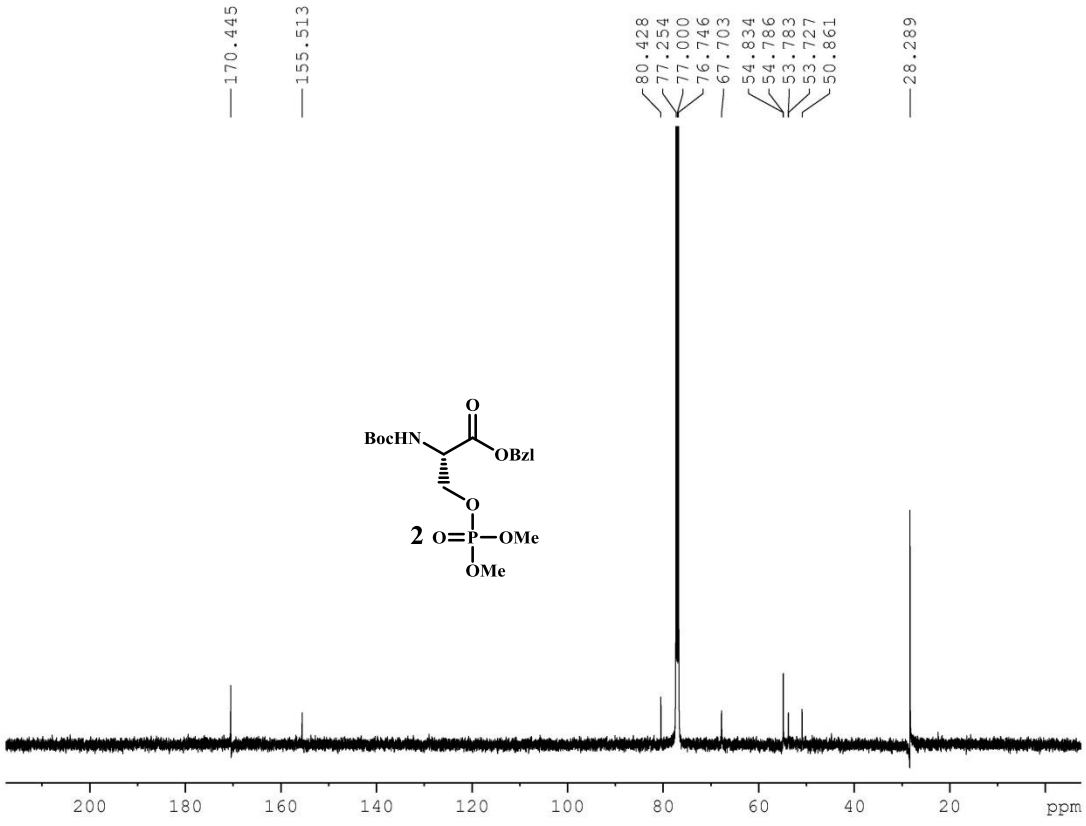
4.309
4.296
4.282
3.805
3.802
3.782
3.780
3.490
1.457
BocHN
OBzl
O
2 O=P—OMe
OMe
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm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80.428
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77.000
76.746
67.703
54.834
54.786
53.783
53.727
50.861
28.289
BocHN
OBzl
O
2 O=P—OMe
OMe
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140
120
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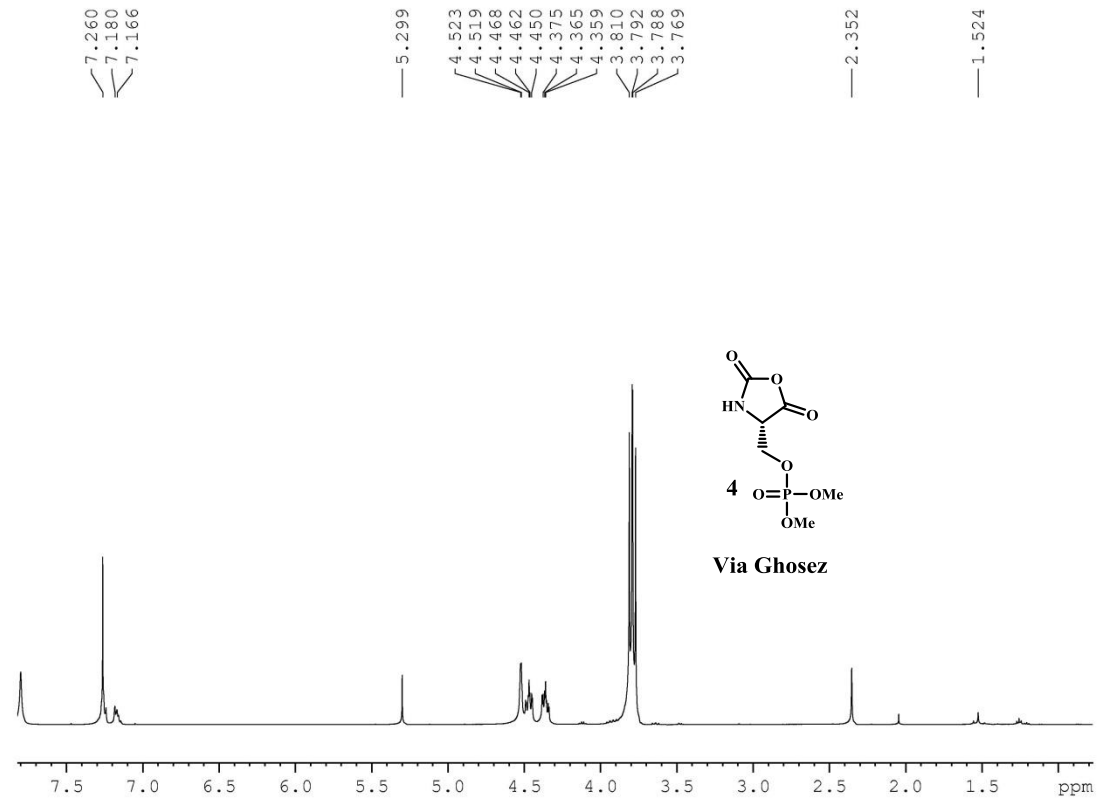
80
60
40
20
ppm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4.519
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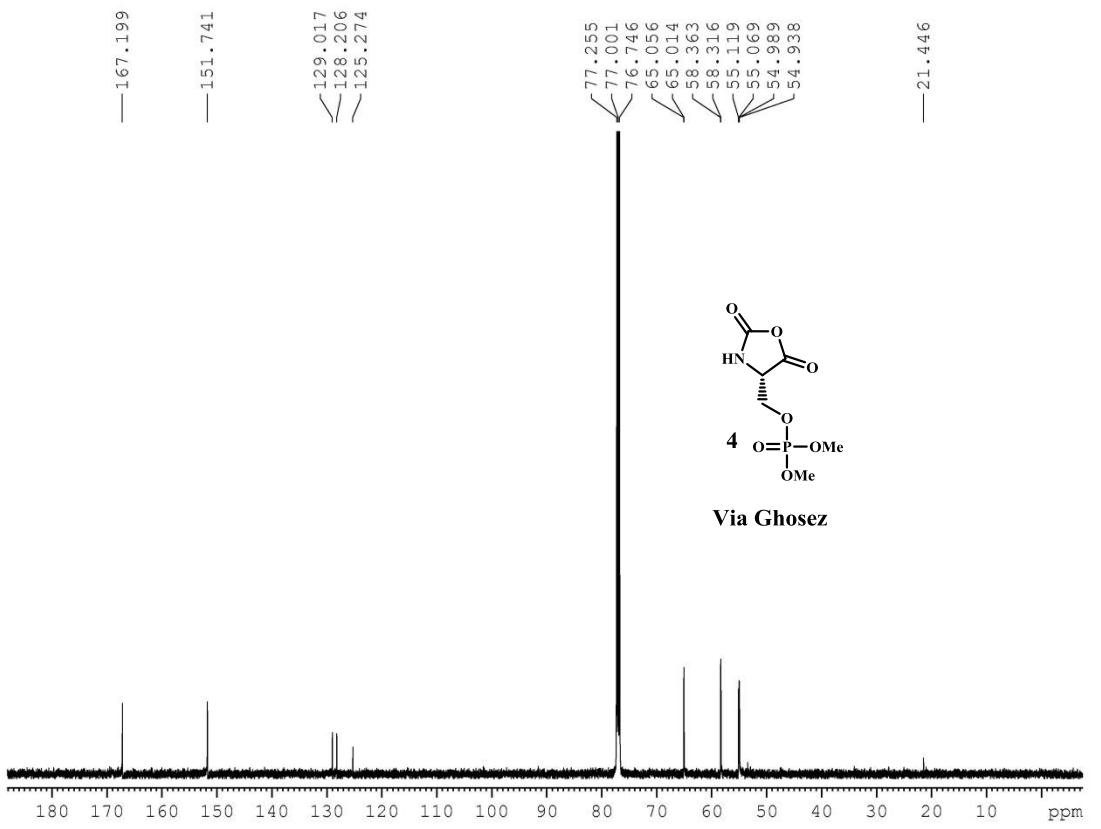
4.462
4.450
4.375
4.365
4.359
3.810
3.792
3.788
3.769
2.352
1.524
4
Via Ghosez
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm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129.017
128.206
125.274
77.255
77.001
76.746
65.056
65.014
58.363
58.316
55.119
55.069
54.989
54.938
21.446
4
Via Ghosez
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150
140
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120
110
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70
60
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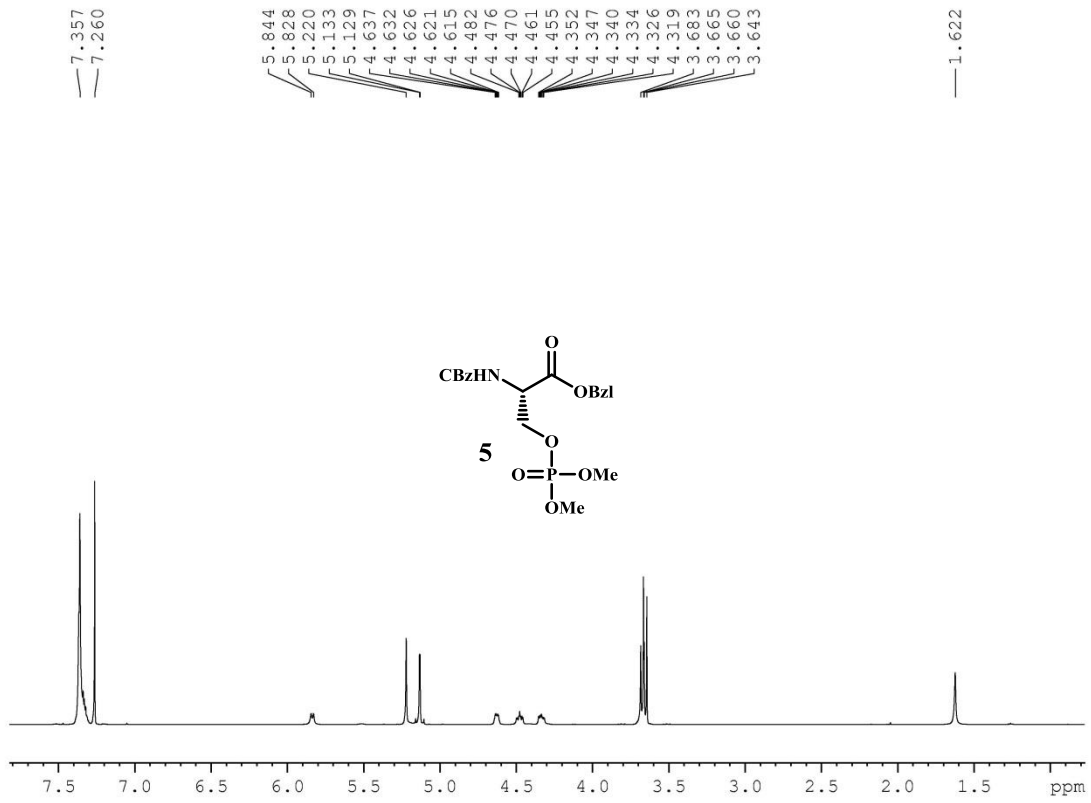
40
30
20
10
ppm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4.455
4.352
4.347
4.340
4.334
4.326
4.319
3.683
3.665
3.660
3.643
1.622
CBzHN
OBzl
O
O
O=P—OMe
OMe
5
7.5
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5.5
5.0
4.5
4.0
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3.0
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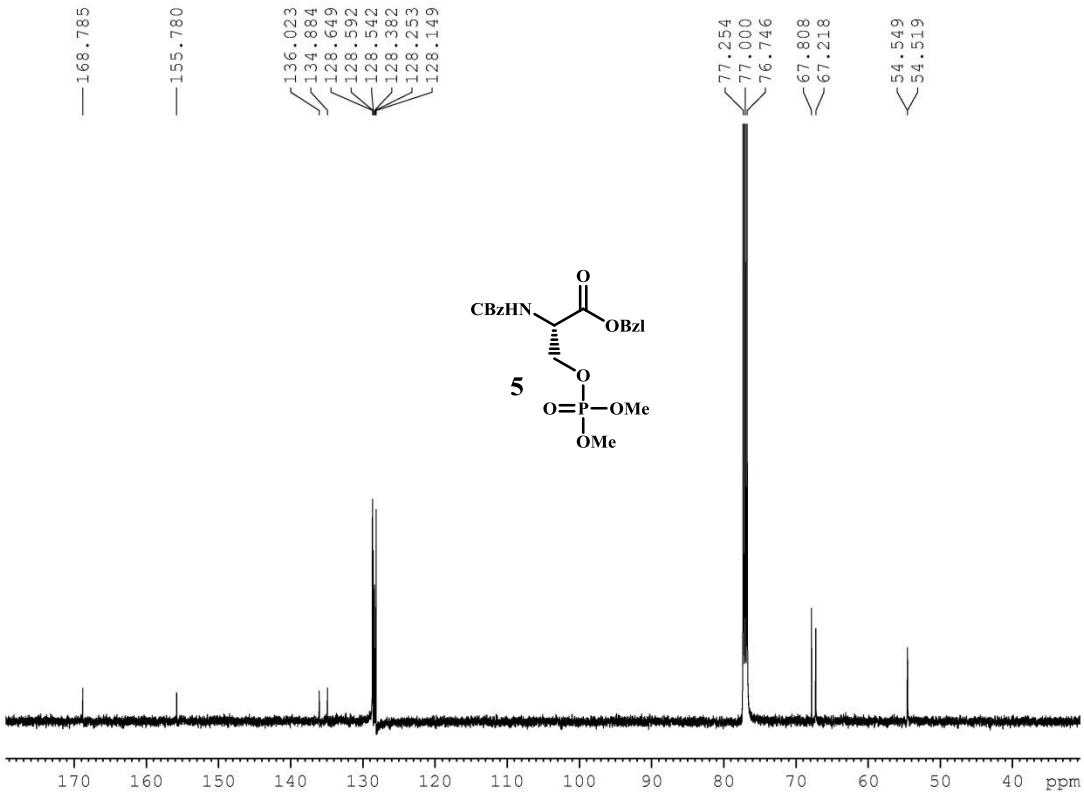
2.0
1.5
ppm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128.592
128.542
128.382
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128.149
77.254
77.000
76.746
67.808
67.218
54.549
54.519
CBzHN
OBzl
O
O
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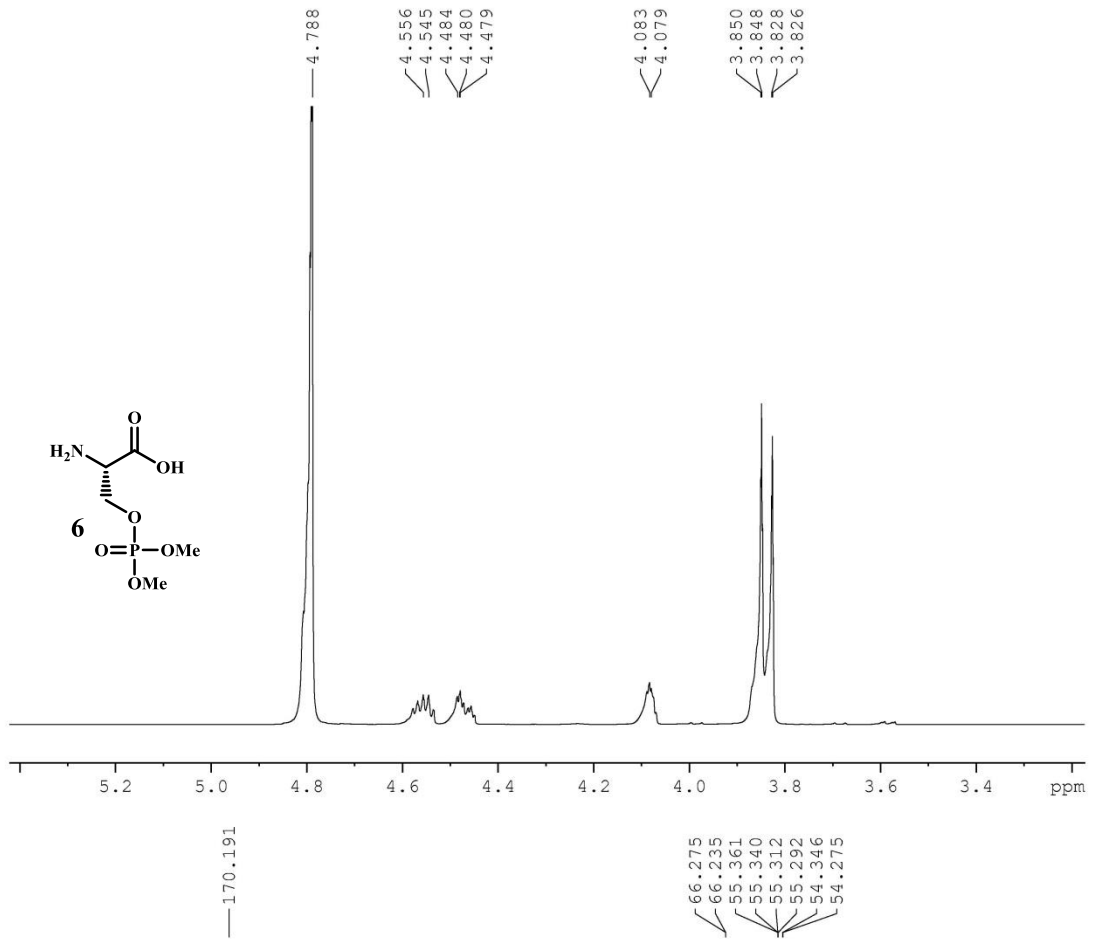
ppm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4.479
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4.079
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3.848
3.828
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5.0
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4.6
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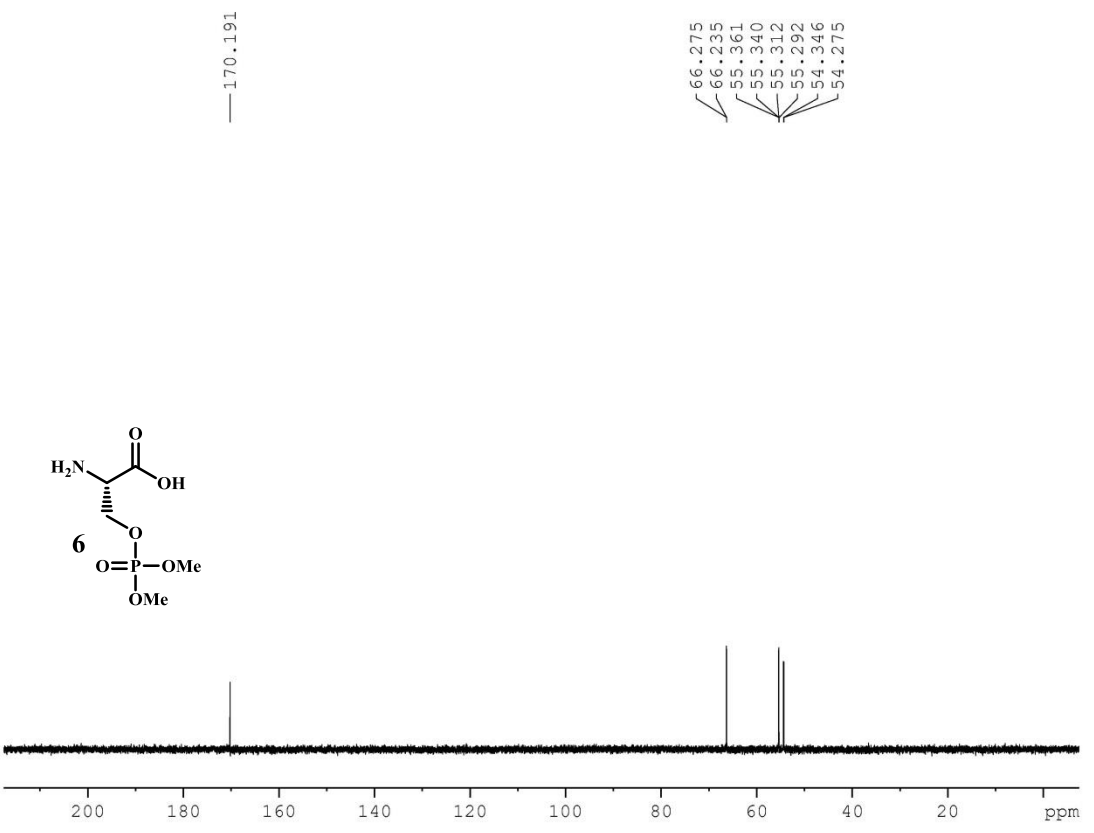
3.6
3.4
ppm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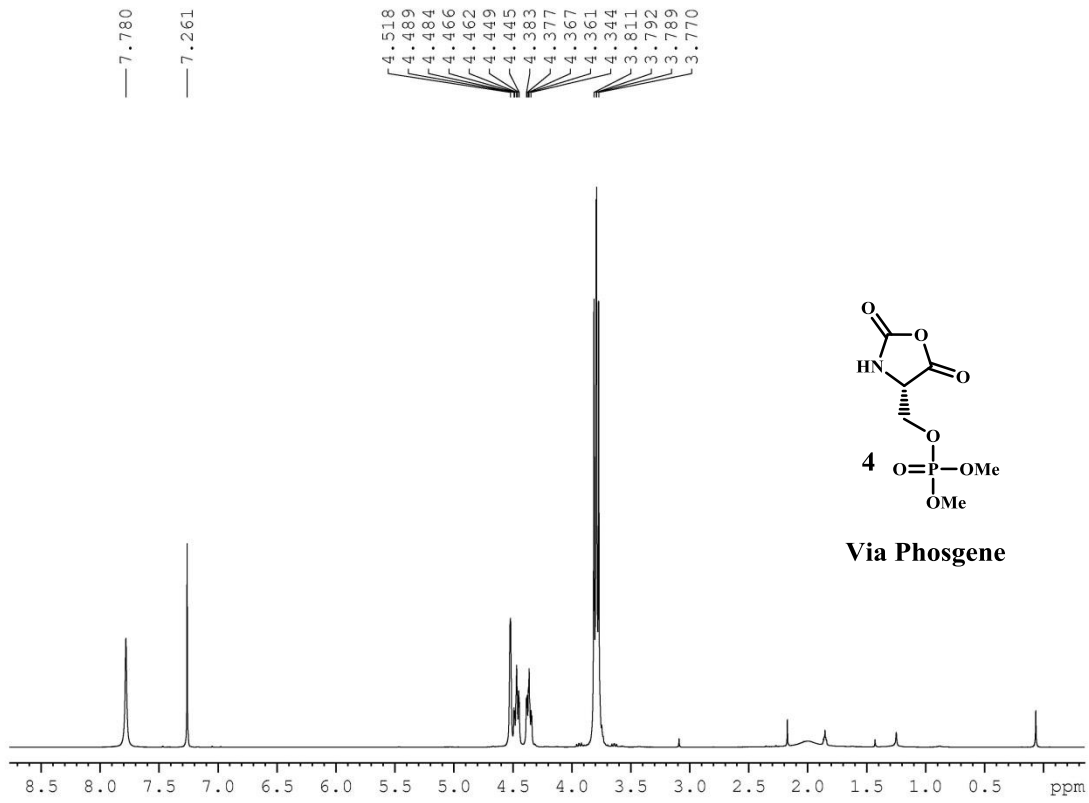
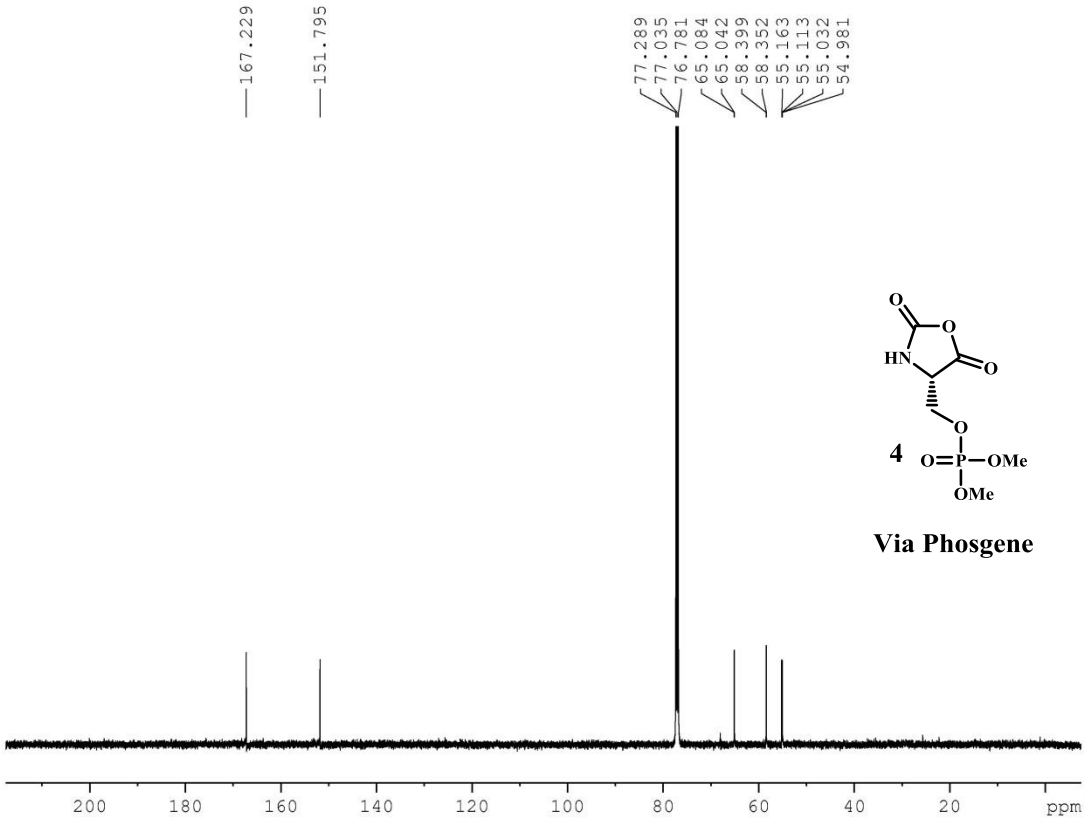
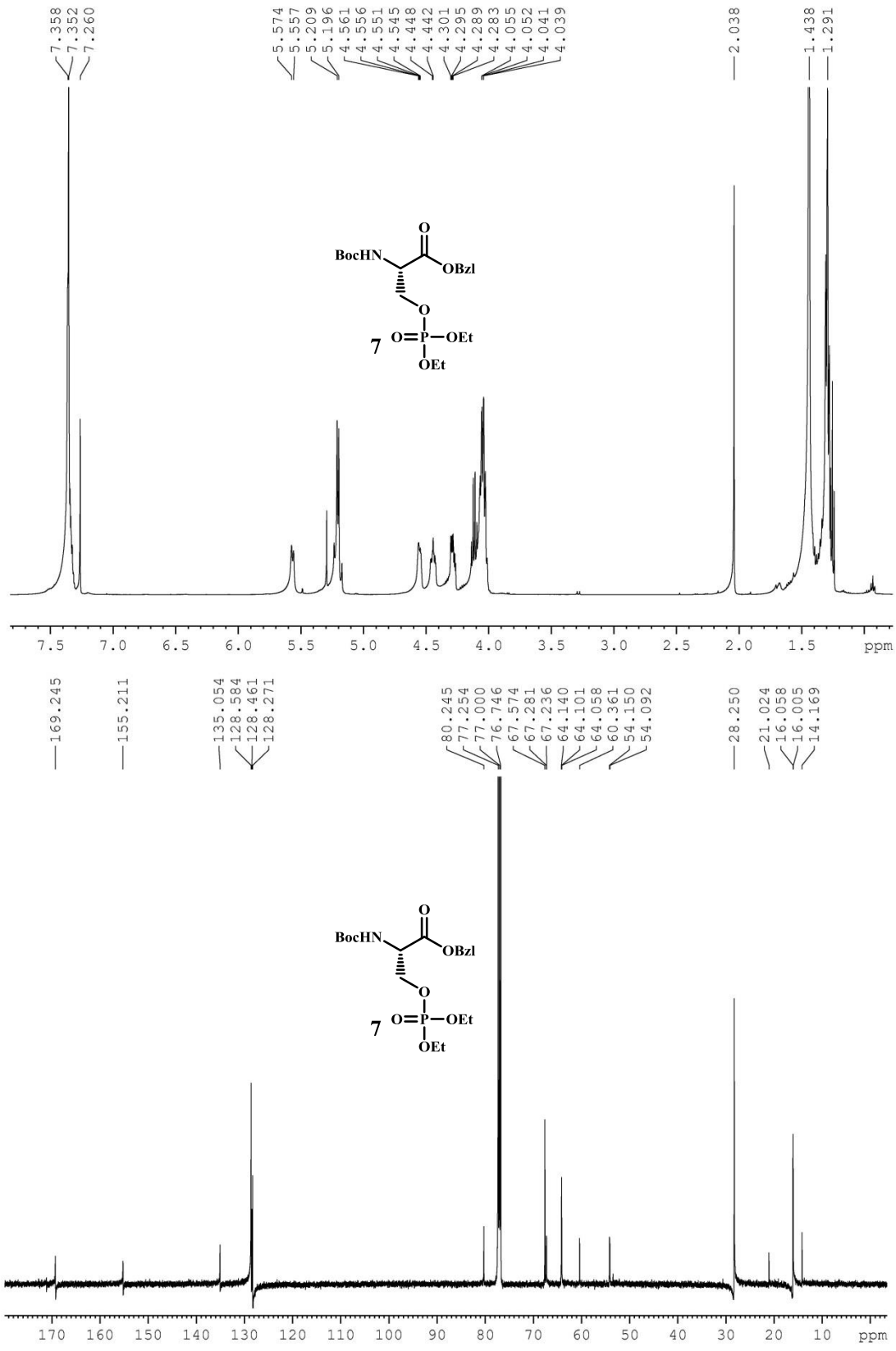


Via Phosgene



Via Phosgene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4.301
4.295
4.289
4.283
4.055
4.052
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4.039
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OBzl
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OEt
7
ppm
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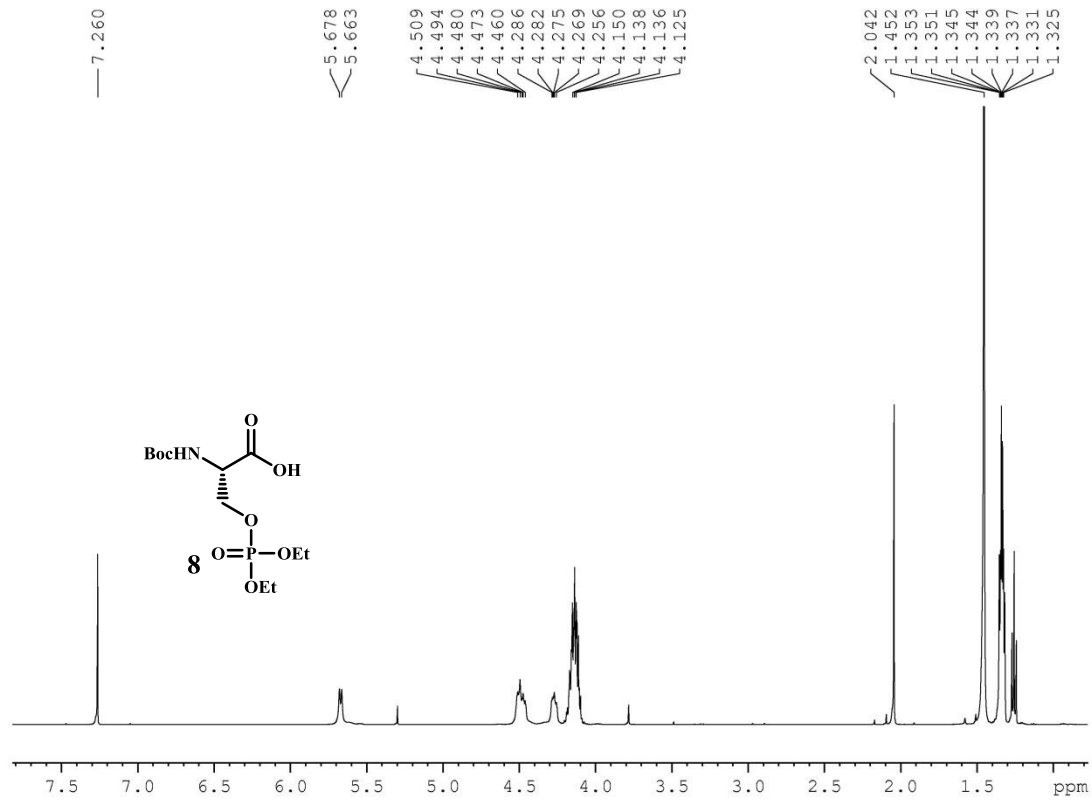
7
ppm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1.351
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OEt
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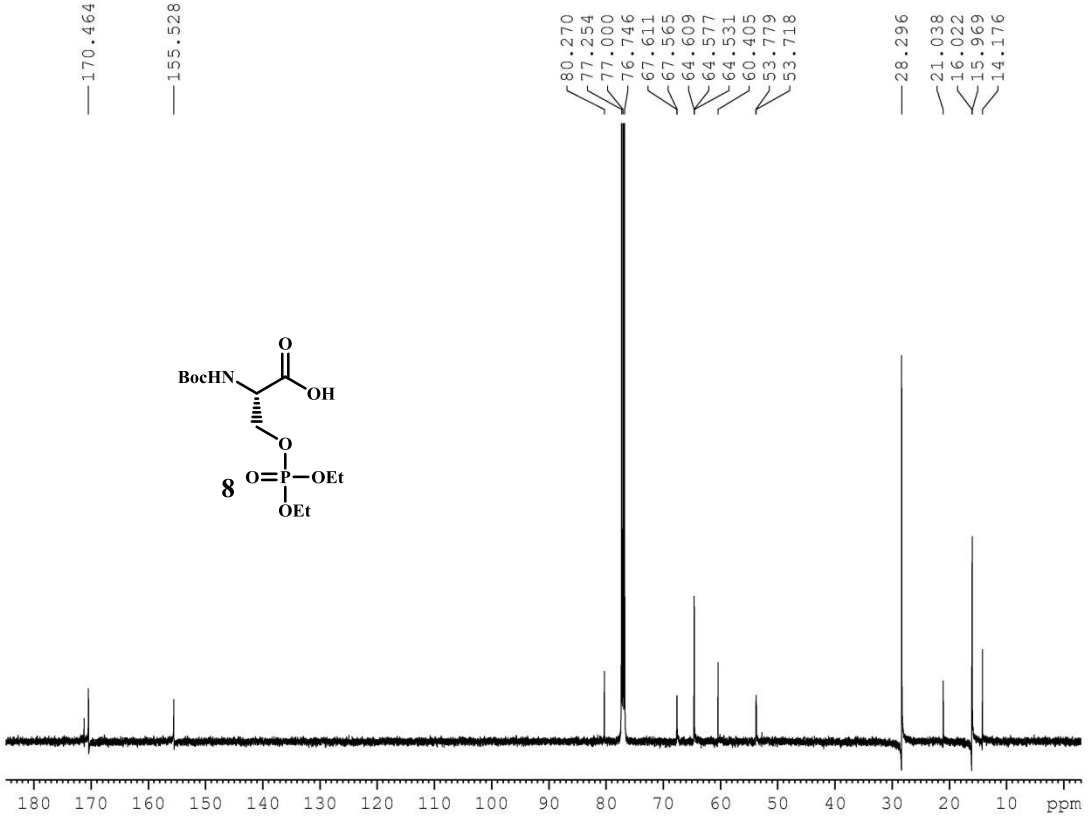
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21.038
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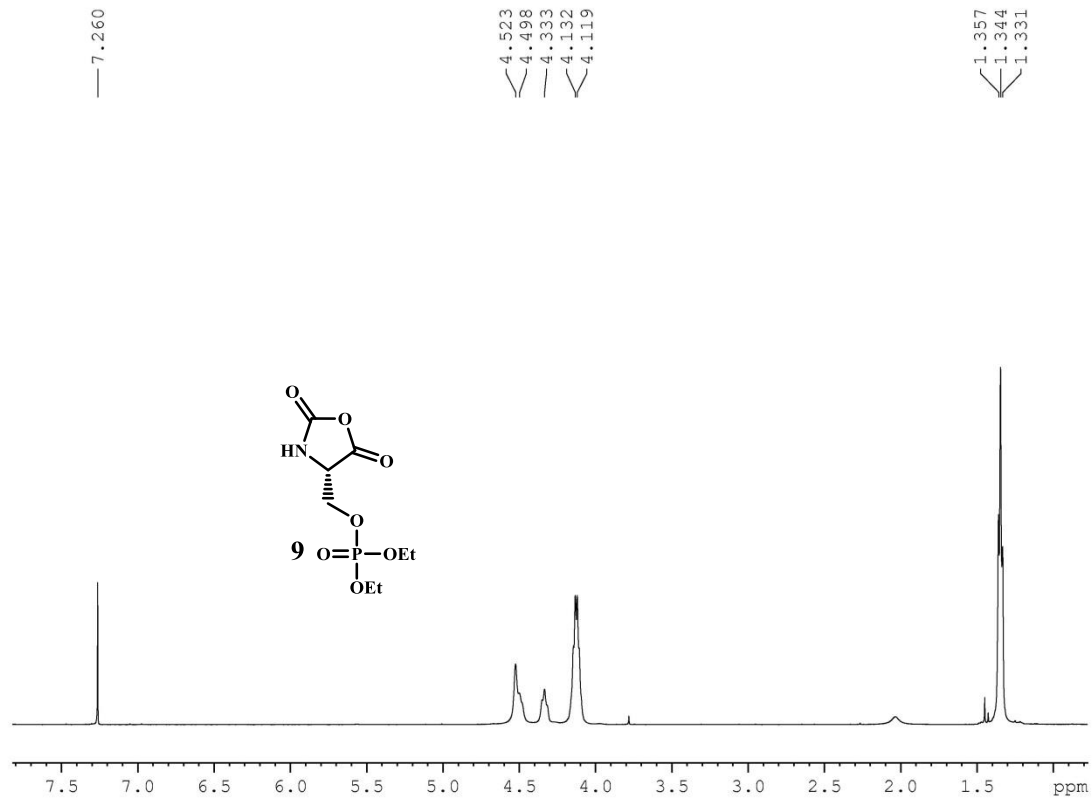
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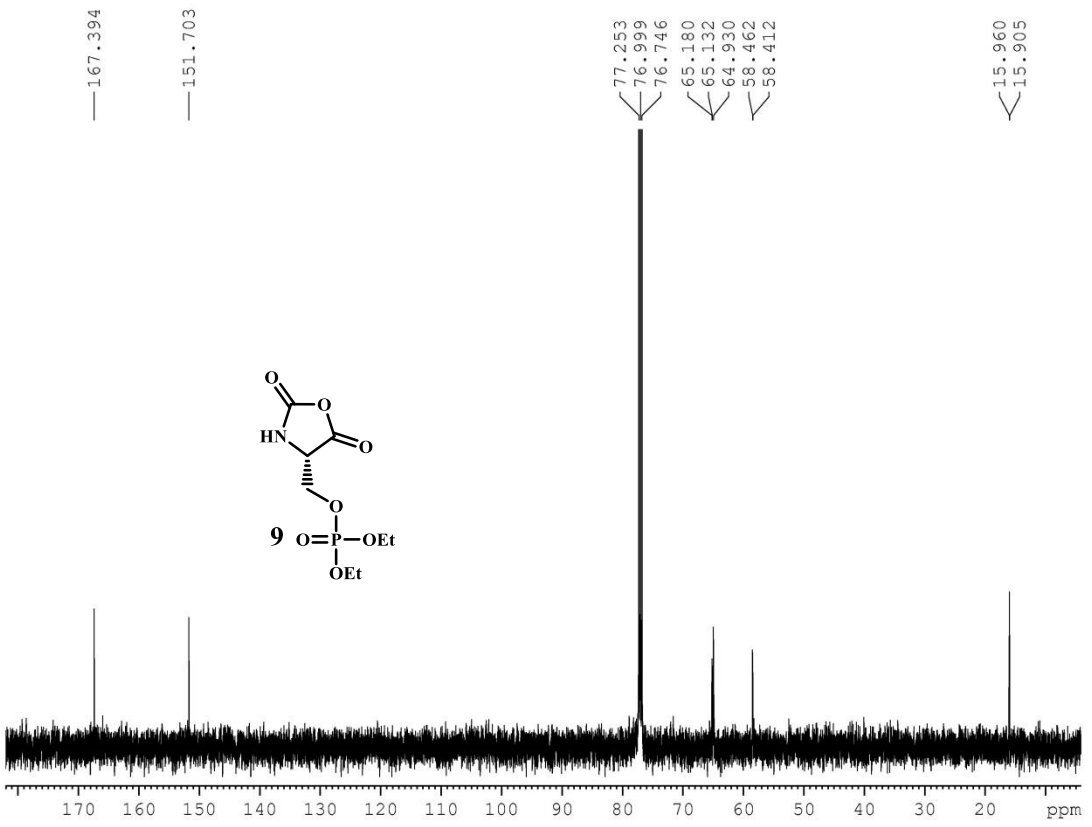
3.5
3.0
2.5
2.0
1.5
ppm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77.253
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64.930
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15.960
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9
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160
150
140
130
120
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100
90
80
70
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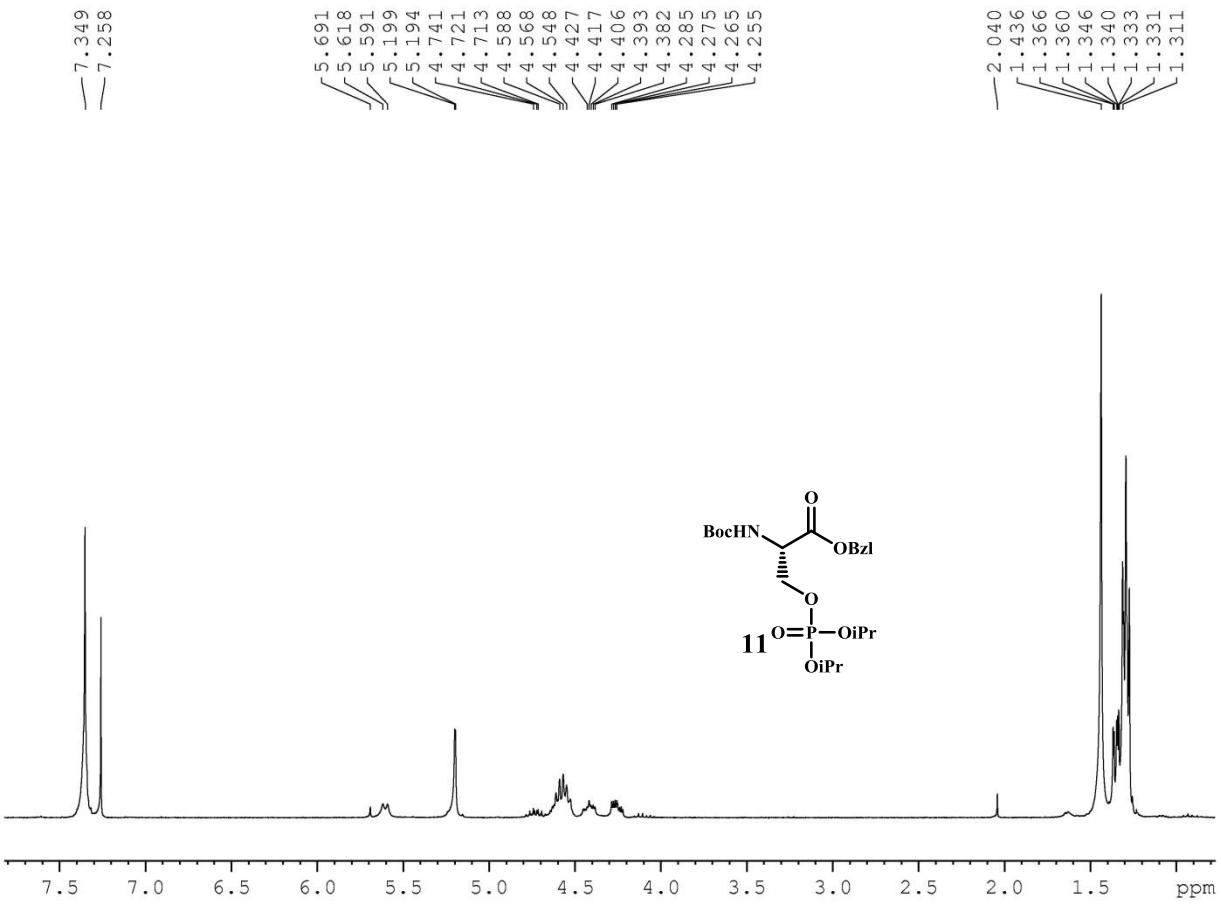
50
40
30
20
ppm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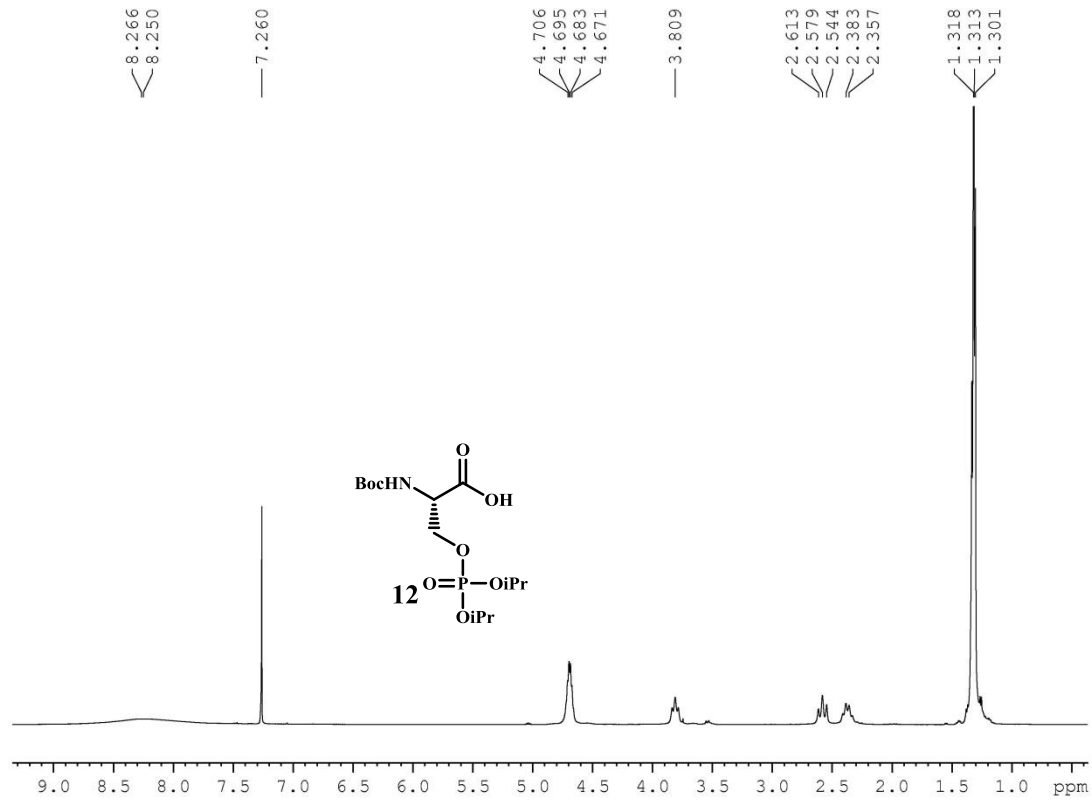
4.568
4.548
4.427
4.417
4.406
4.393
4.382
4.285
4.275
4.265
4.255
2.040
1.436
1.366
1.360
1.346
1.340
1.333
1.331
1.311
BocHN
OBzl
O
O
O=P—OiPr
OiPr
11
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm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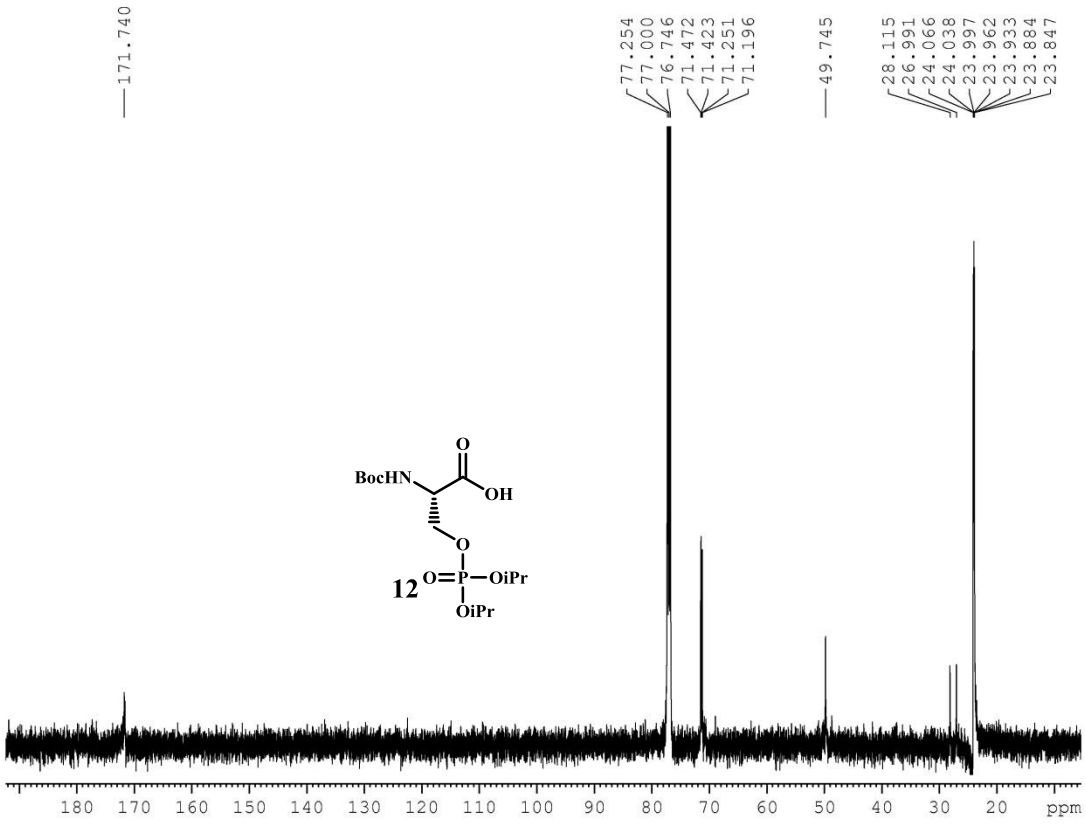
8.250
7.260
4.706
4.695
4.683
4.671
3.809
2.613
2.579
2.544
2.383
2.357
1.318
1.313
1.301
BocHN
OH
O
O=P—OiPr
OiPr
12
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm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77.000
76.746
71.472
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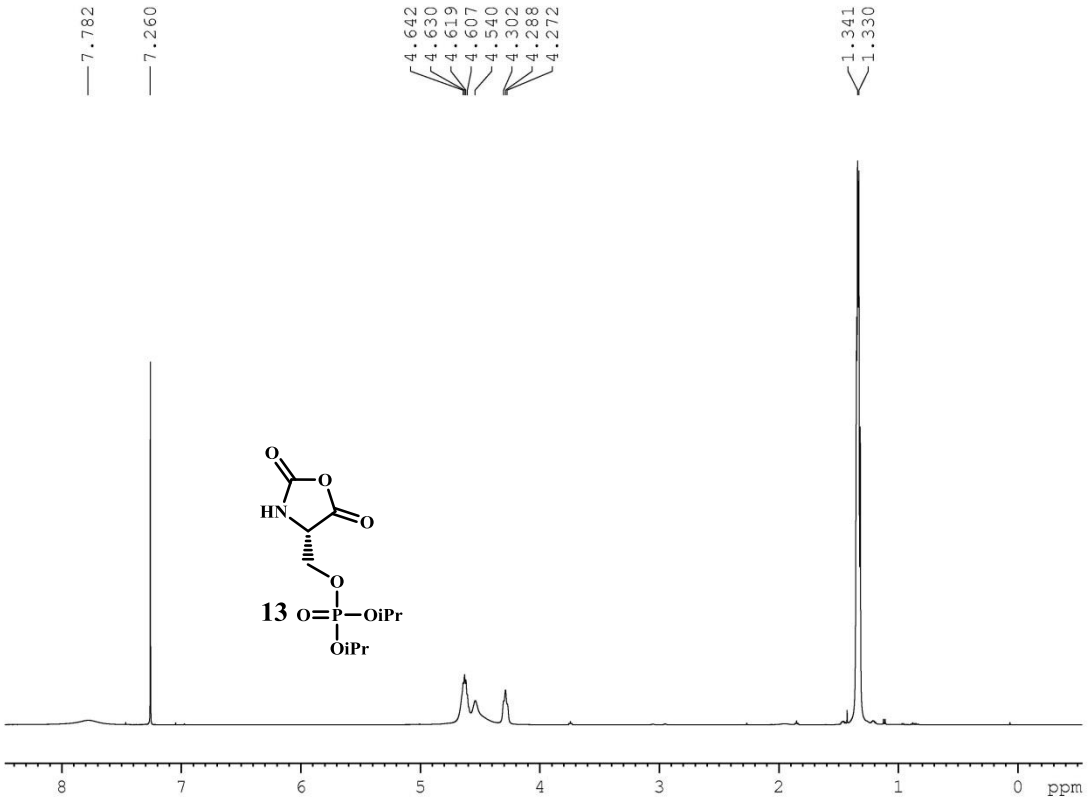
71.251
71.196
49.745
28.115
26.991
24.066
24.038
23.997
23.962
23.933
23.884
23.847
BocHN
OH
O
O=P—OiPr
OiPr
12
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm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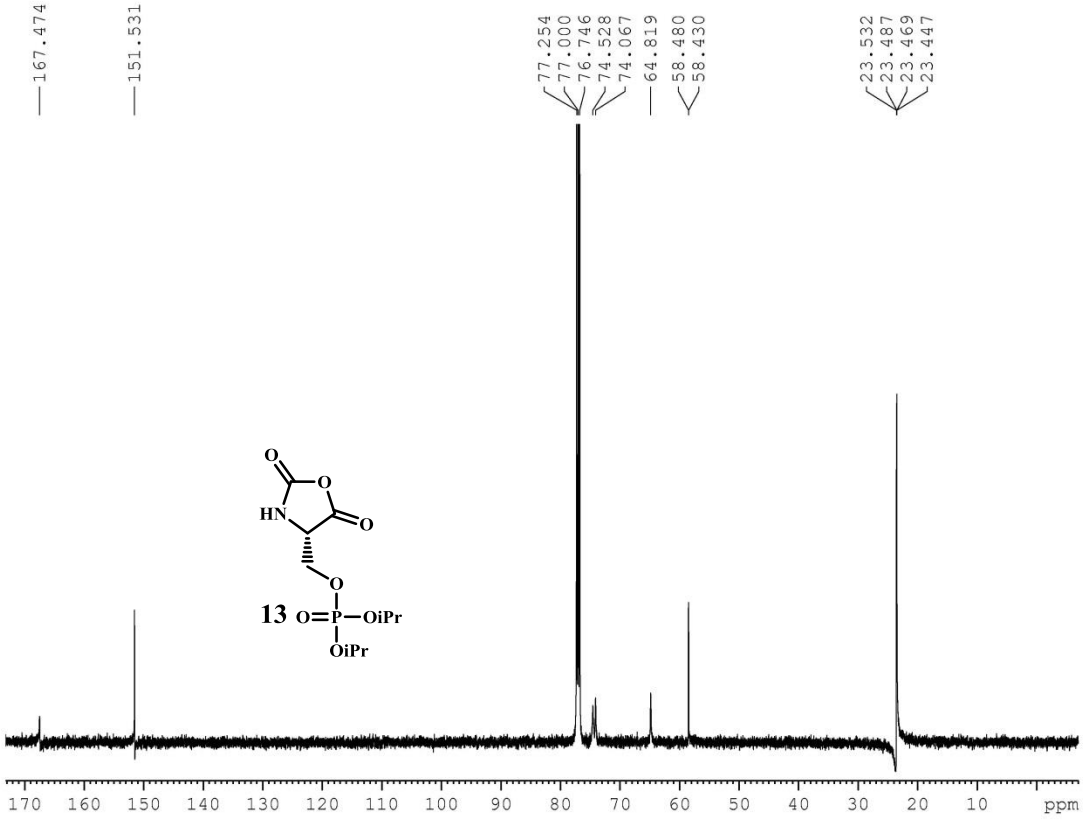
4.630
4.619
4.607
4.540
4.302
4.288
4.272
1.341
1.330
13
OiPr
OiPr
8
7
6
5
4
3
2
1
0
ppm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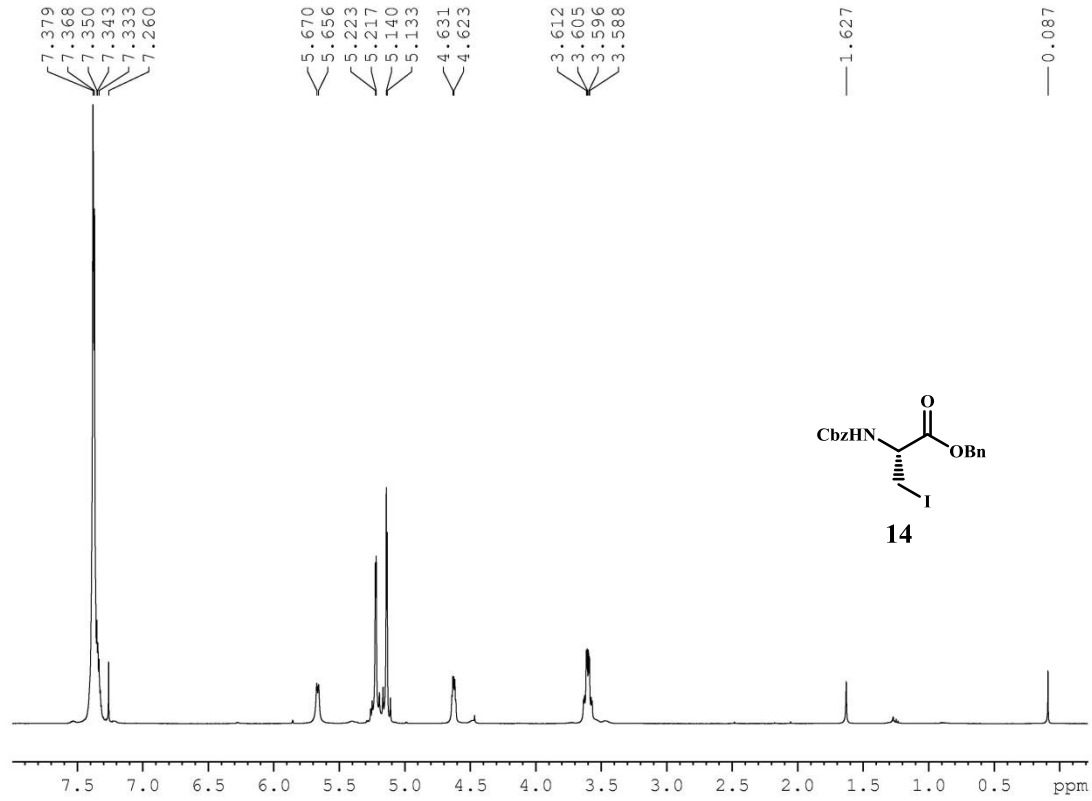
151.531
77.254
77.000
76.746
74.528
74.067
64.819
58.480
58.430
23.532
23.487
23.469
23.447
13
OiPr
OiPr
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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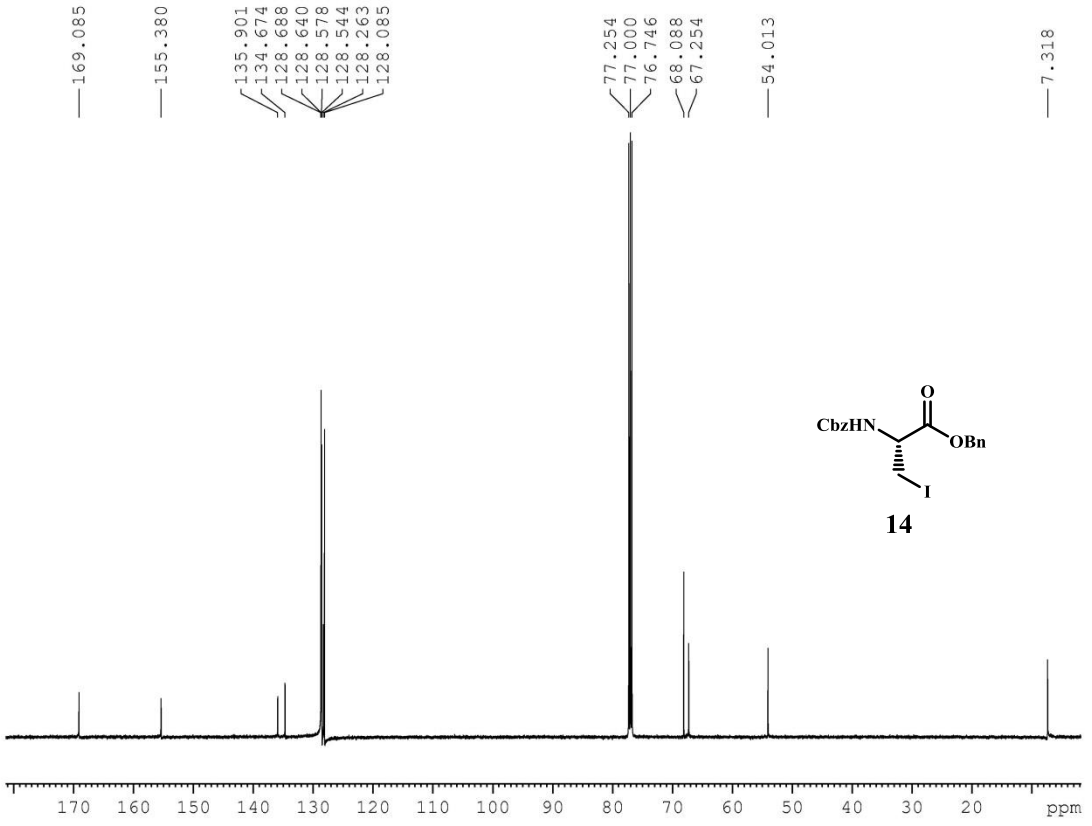
7.350
7.343
7.333
7.260
5.670
5.656
5.223
5.217
5.140
5.133
4.631
4.623
3.612
3.605
3.596
3.588
1.627
0.087
CbzHN
O
OBn
I
14
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm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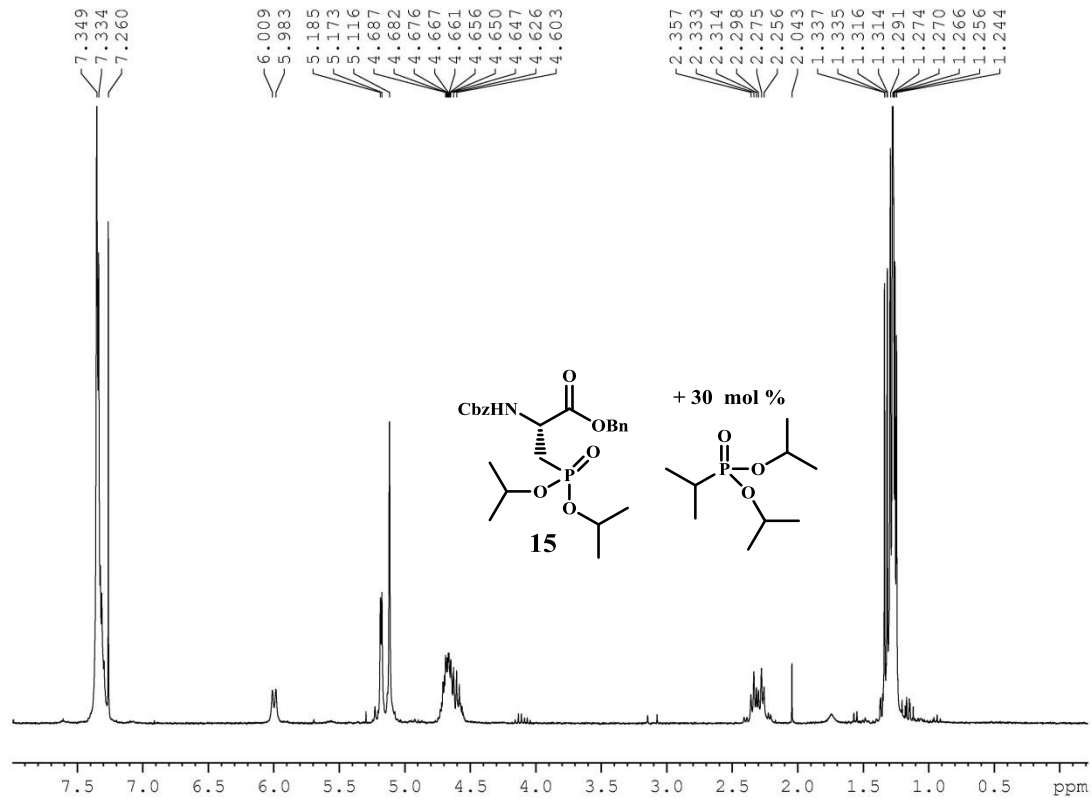
155.380
135.901
134.674
128.688
128.640
128.578
128.544
128.263
128.085
77.254
77.000
76.746
68.088
67.254
54.013
7.318
CbzHN
O
OBn
I
14
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm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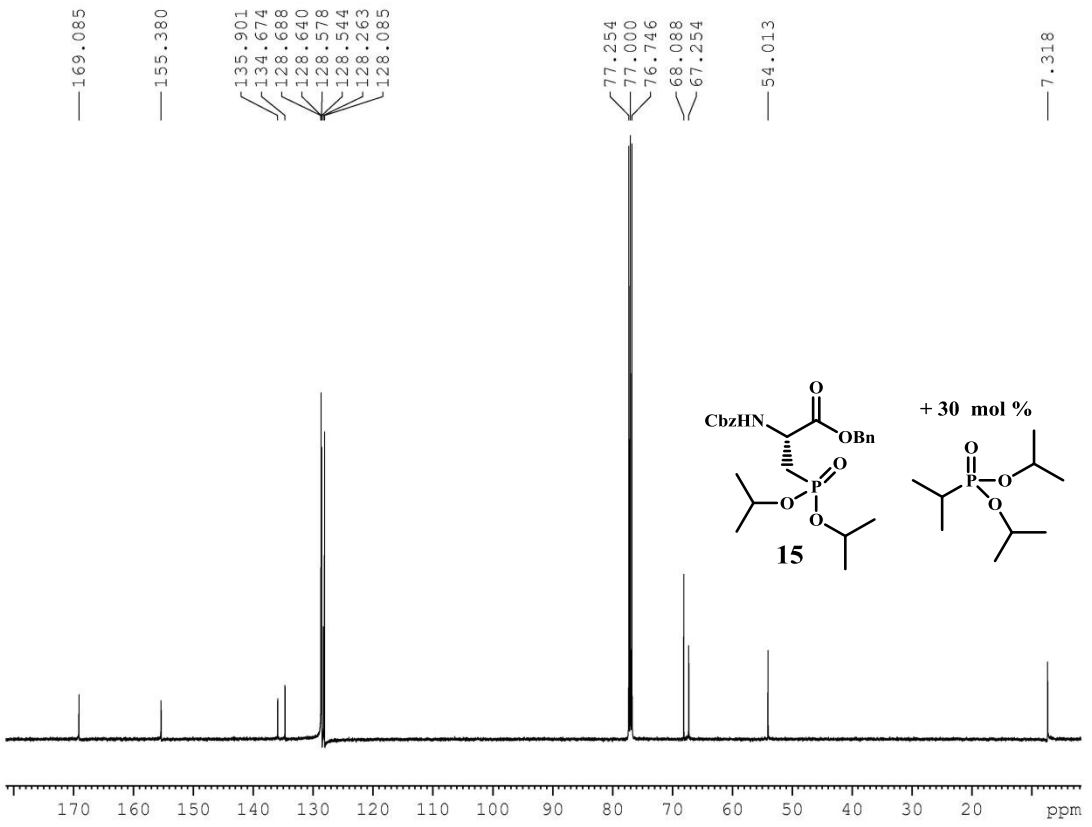
7.334
7.260
6.009
5.983
5.185
5.173
5.116
4.687
4.682
4.676
4.667
4.661
4.656
4.650
4.647
4.626
4.603
2.357
2.333
2.314
2.298
2.275
2.256
2.043
1.337
1.335
1.316
1.314
1.291
1.274
1.270
1.266
1.256
1.244
CbzHN
OBn
15
+ 30 mol %
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm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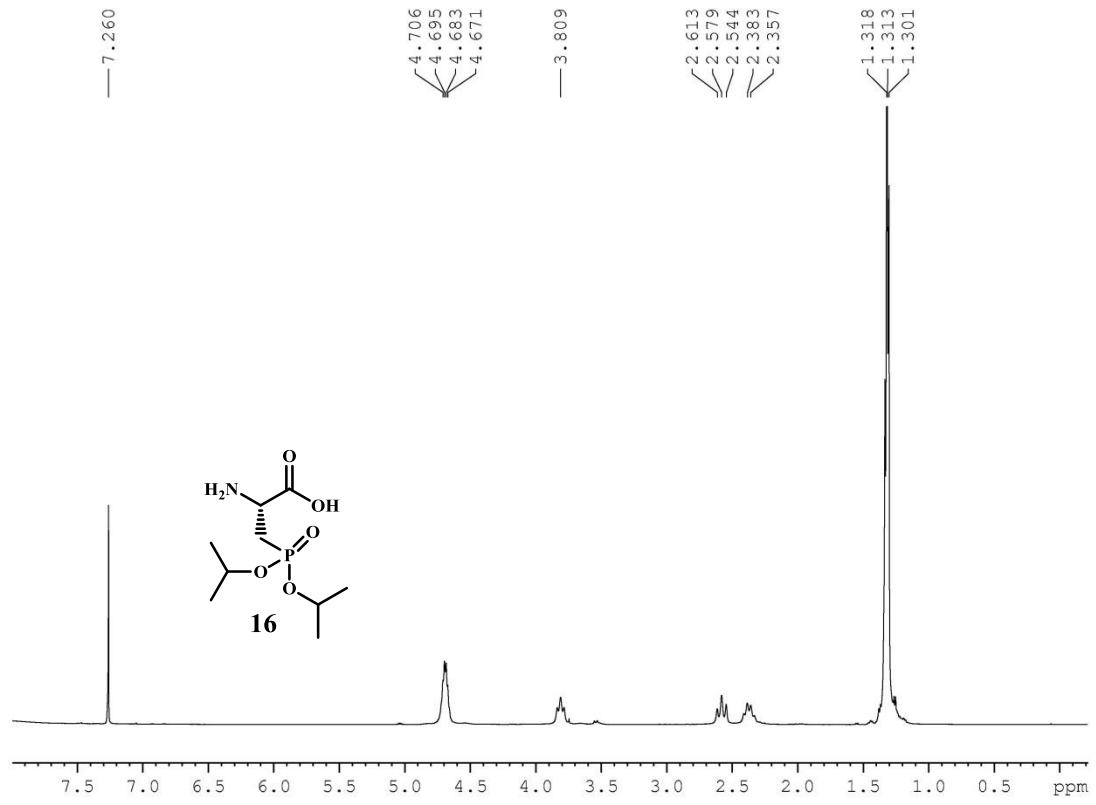
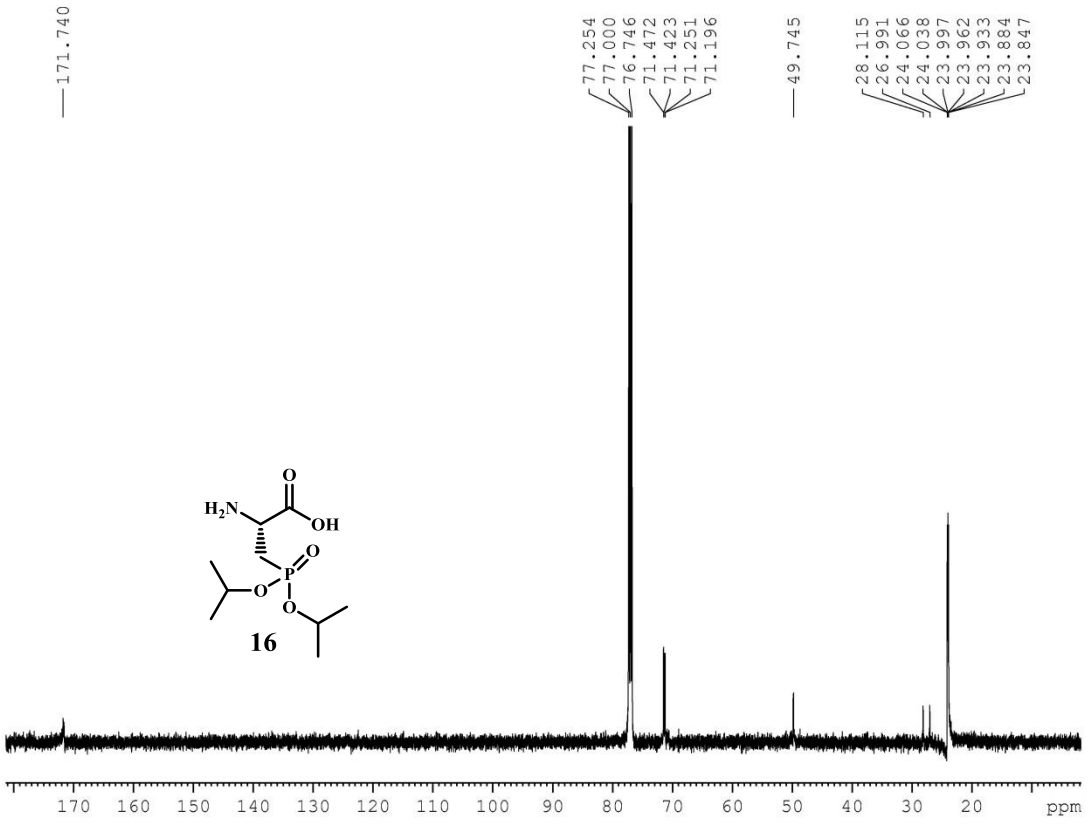
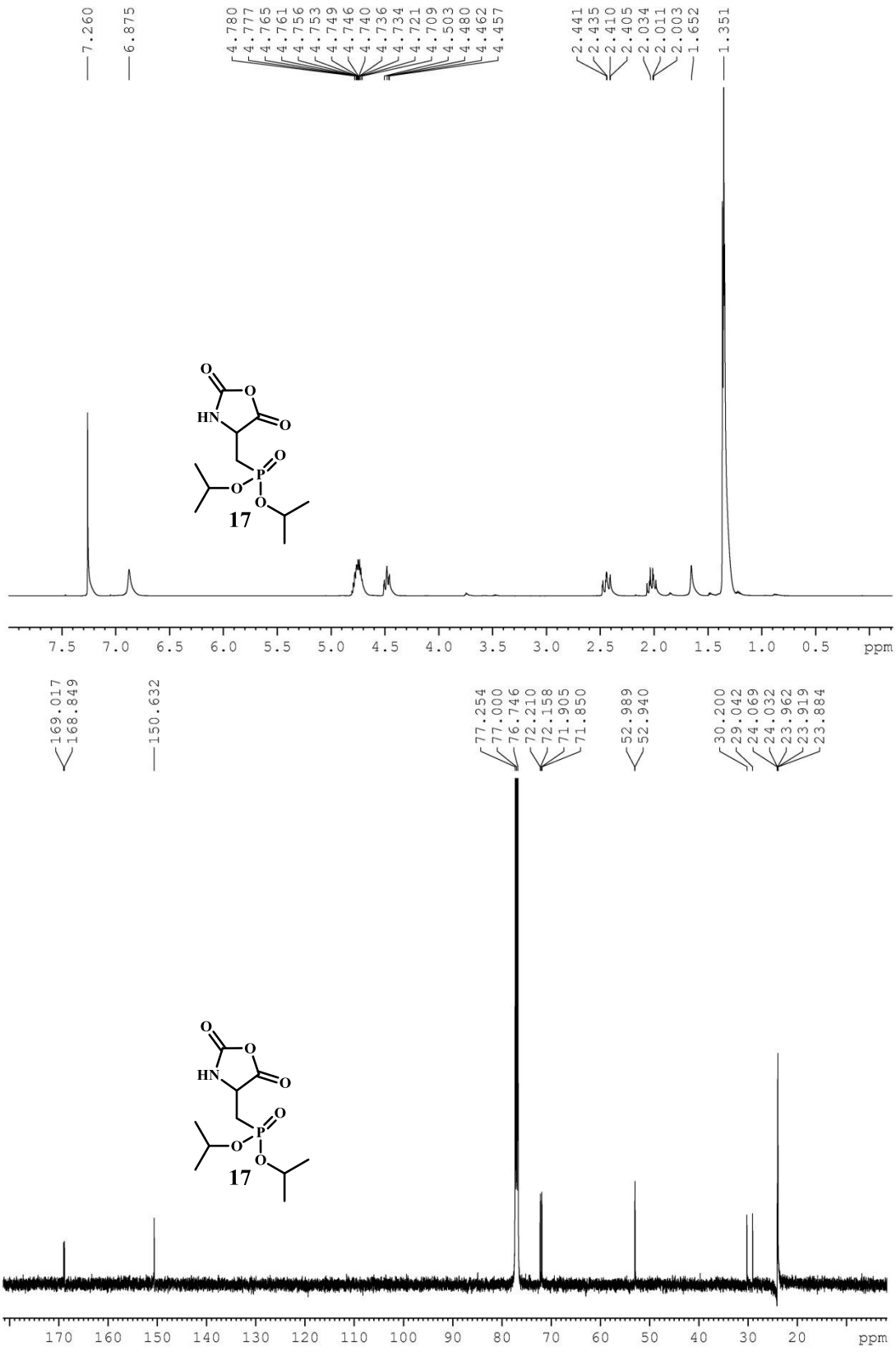
155.380
135.901
134.674
128.688
128.640
128.578
128.544
128.263
128.085
77.254
77.000
76.746
68.088
67.254
54.013
7.318
CbzHN
OBn
15
+ 30 mol %
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


7.260
4.706
4.695
4.683
4.671
3.809
2.613
2.579
2.544
2.383
2.357
1.318
1.313
1.301
16
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm


171.740
77.254
77.000
76.746
71.472
71.423
71.251
71.196
49.745
28.115
26.991
24.066
24.038
23.997
23.962
23.933
23.884
23.847
16
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm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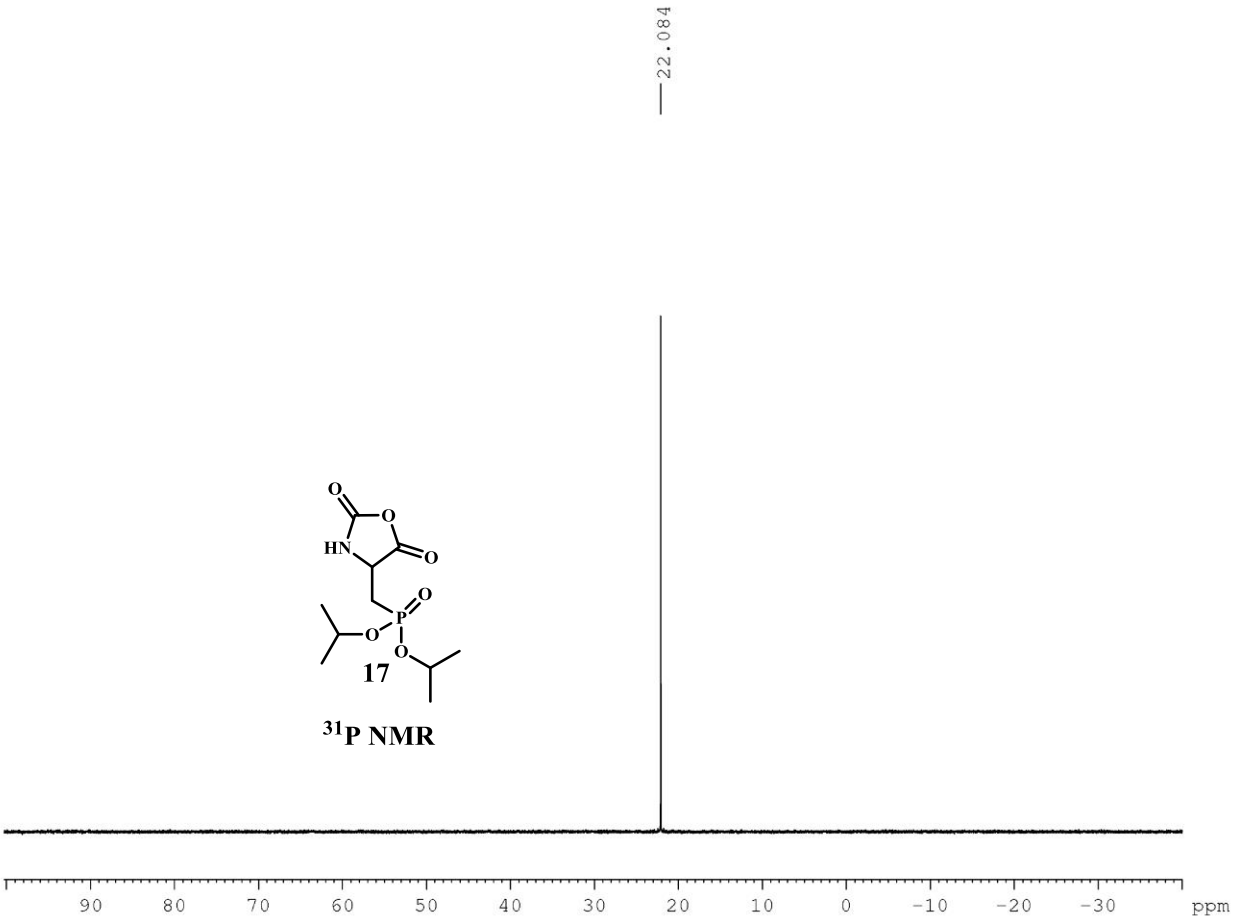
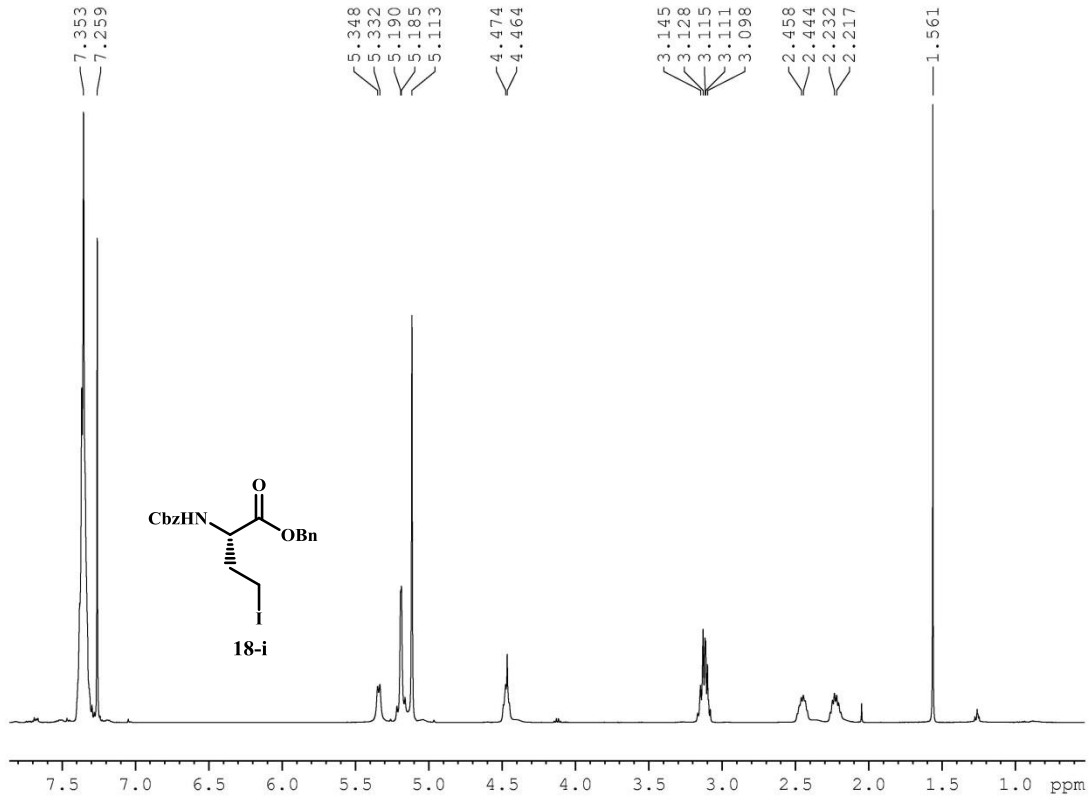
4.780
4.777
4.765
4.761
4.756
4.753
4.749
4.746
4.740
4.736
4.734
4.721
4.709
4.503
4.480
4.462
4.457
2.441
2.435
2.410
2.405
2.034
2.011
2.003
1.652
1.351
17
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
169.017
168.849
150.632
77.254
77.000
76.746
72.210
72.158
71.905
71.850
52.989
52.940
30.200
29.042
24.069
24.032
23.962
23.919
23.884
17
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


22.084
O
O
HN
O
O
P
O
O
17
31P NMR
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
ppm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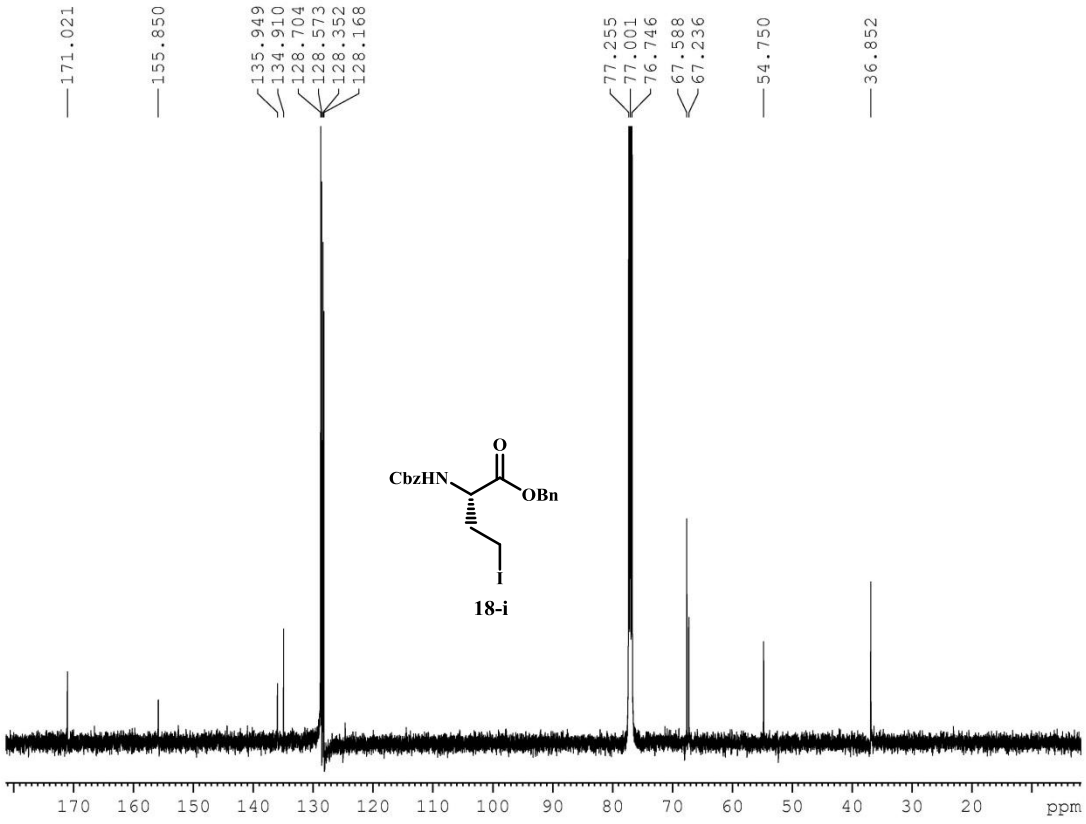
7.259
5.348
5.332
5.190
5.185
5.113
4.474
4.464
3.145
3.128
3.115
3.111
3.098
2.458
2.444
2.232
2.217
1.561
CbzHN
O
OBn
I
18-i
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm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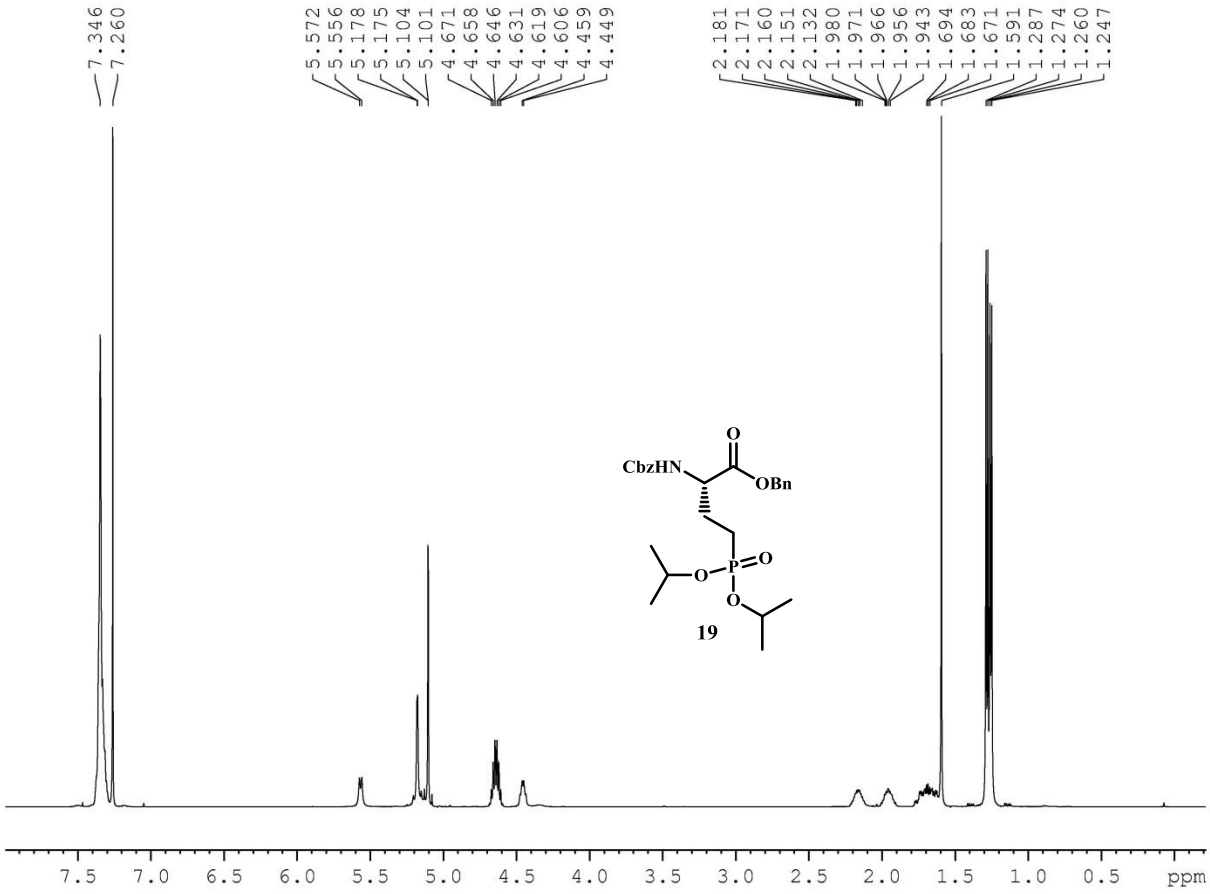
134.910
128.704
128.573
128.352
128.168
77.255
77.001
76.746
67.588
67.236
54.750
36.852
CbzHN
O
OBn
I
18-i
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm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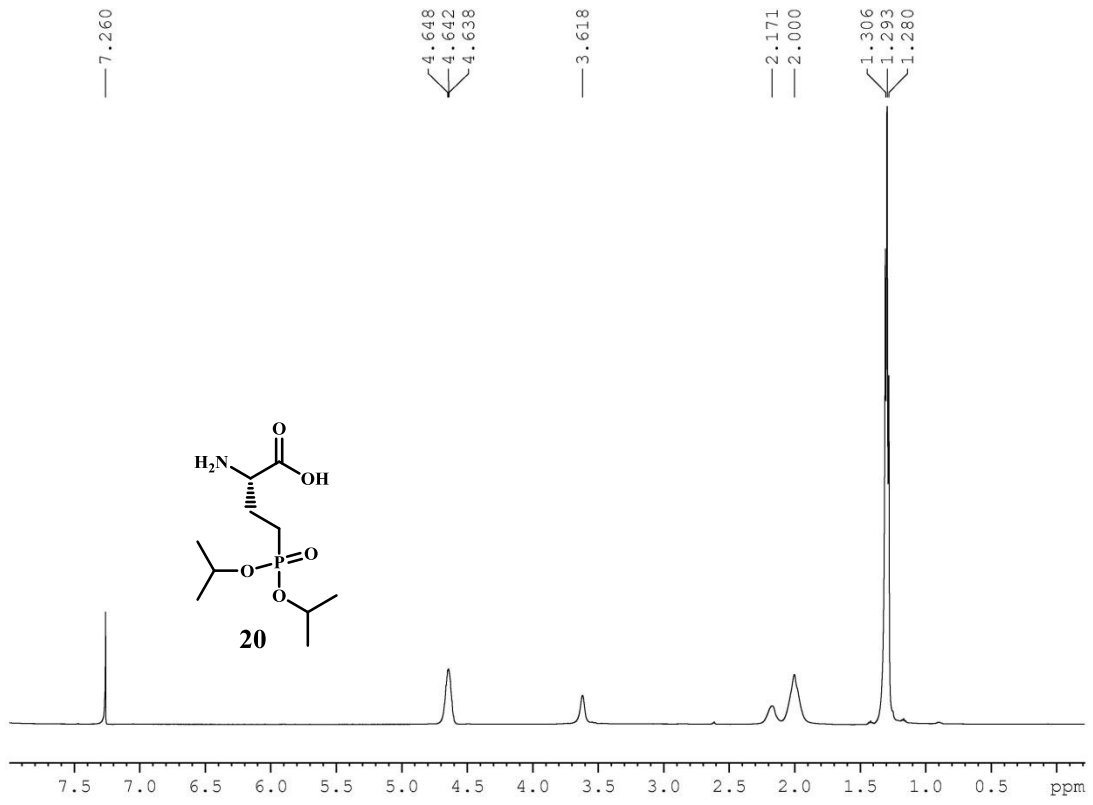
5.175
5.104
5.101
4.671
4.658
4.646
4.631
4.619
4.606
4.459
4.449
2.181
2.171
2.160
2.151
2.132
1.980
1.971
1.966
1.956
1.943
1.694
1.683
1.671
1.591
1.287
1.274
1.260
1.247
CbzHN
OBn
O
P
19
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm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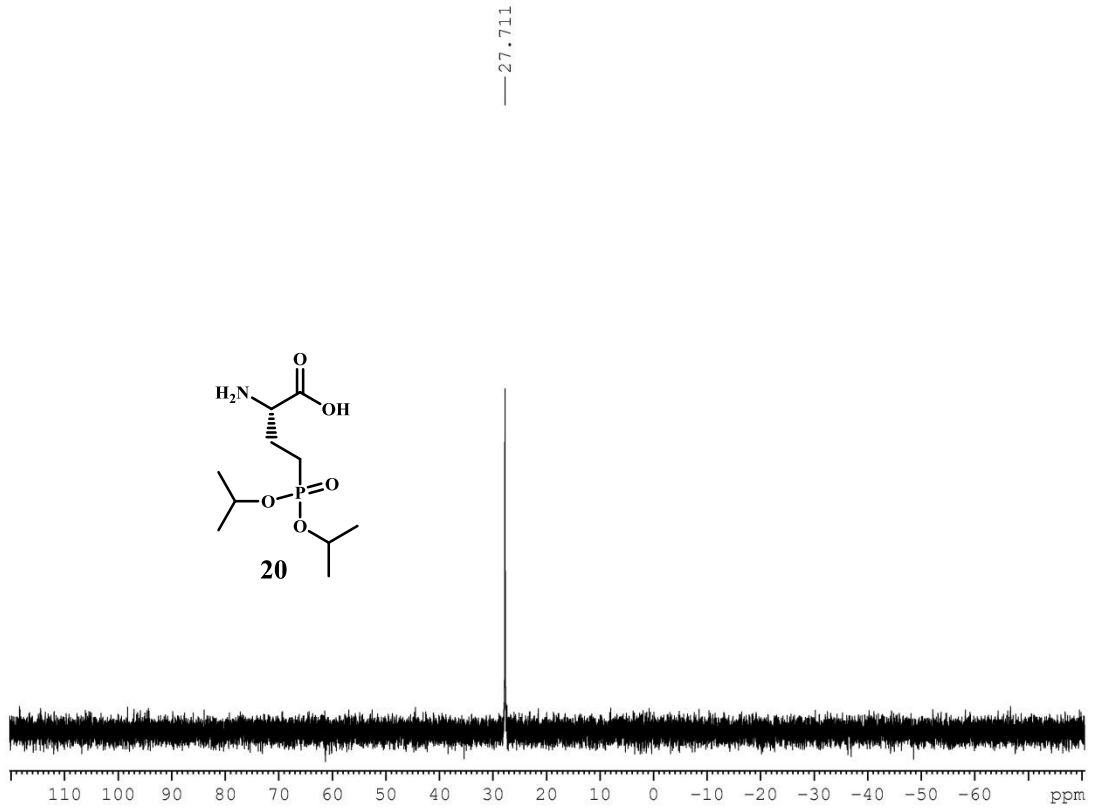
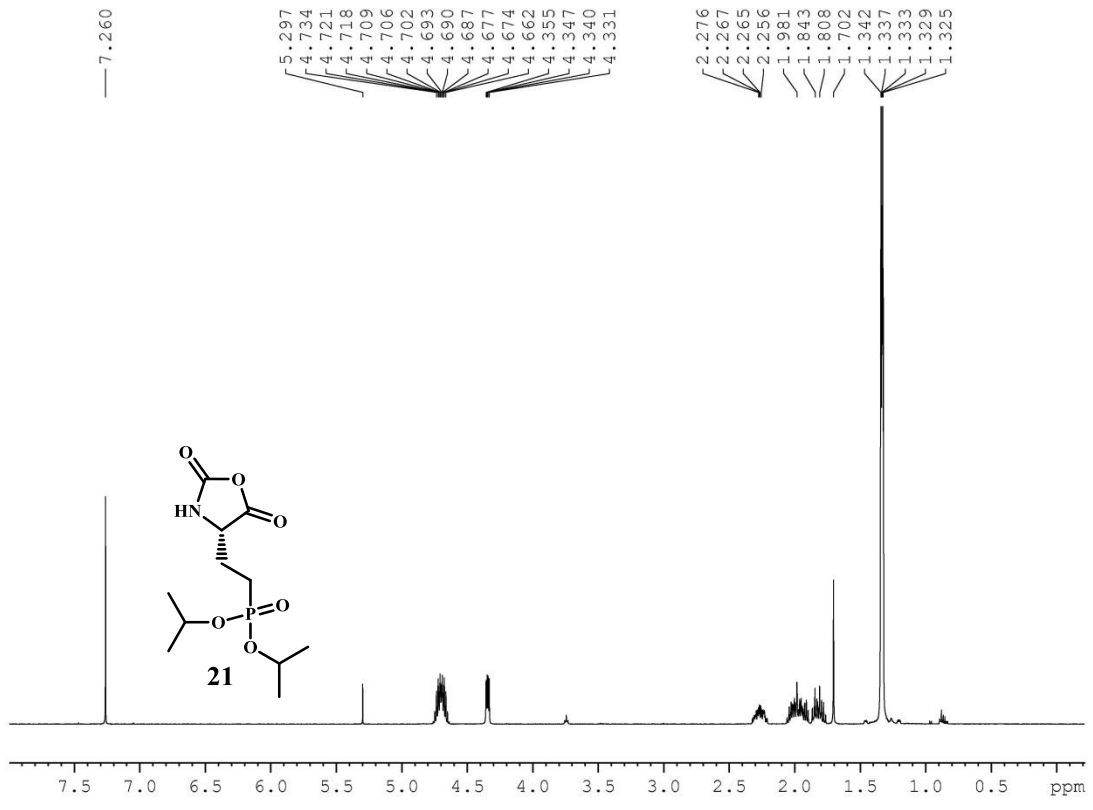
4.648
4.642
4.638
3.618
2.171
2.000
1.306
1.293
1.280
20
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm


27.711
20
110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 ppm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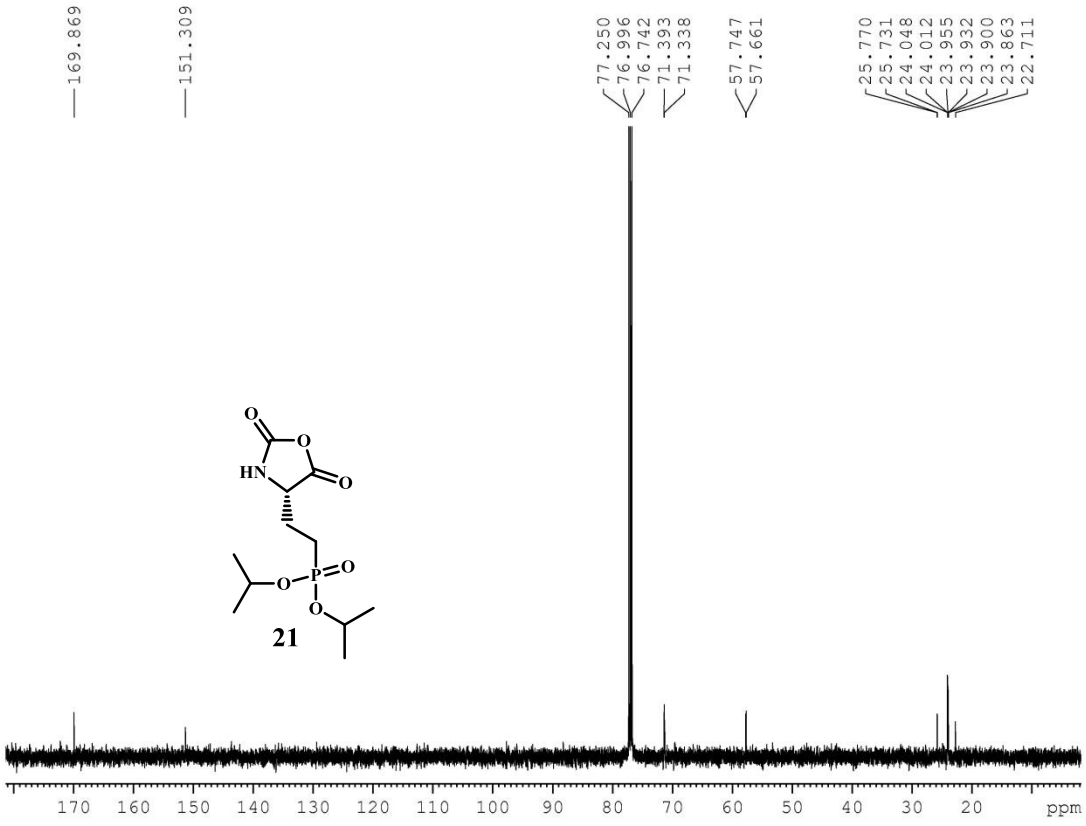
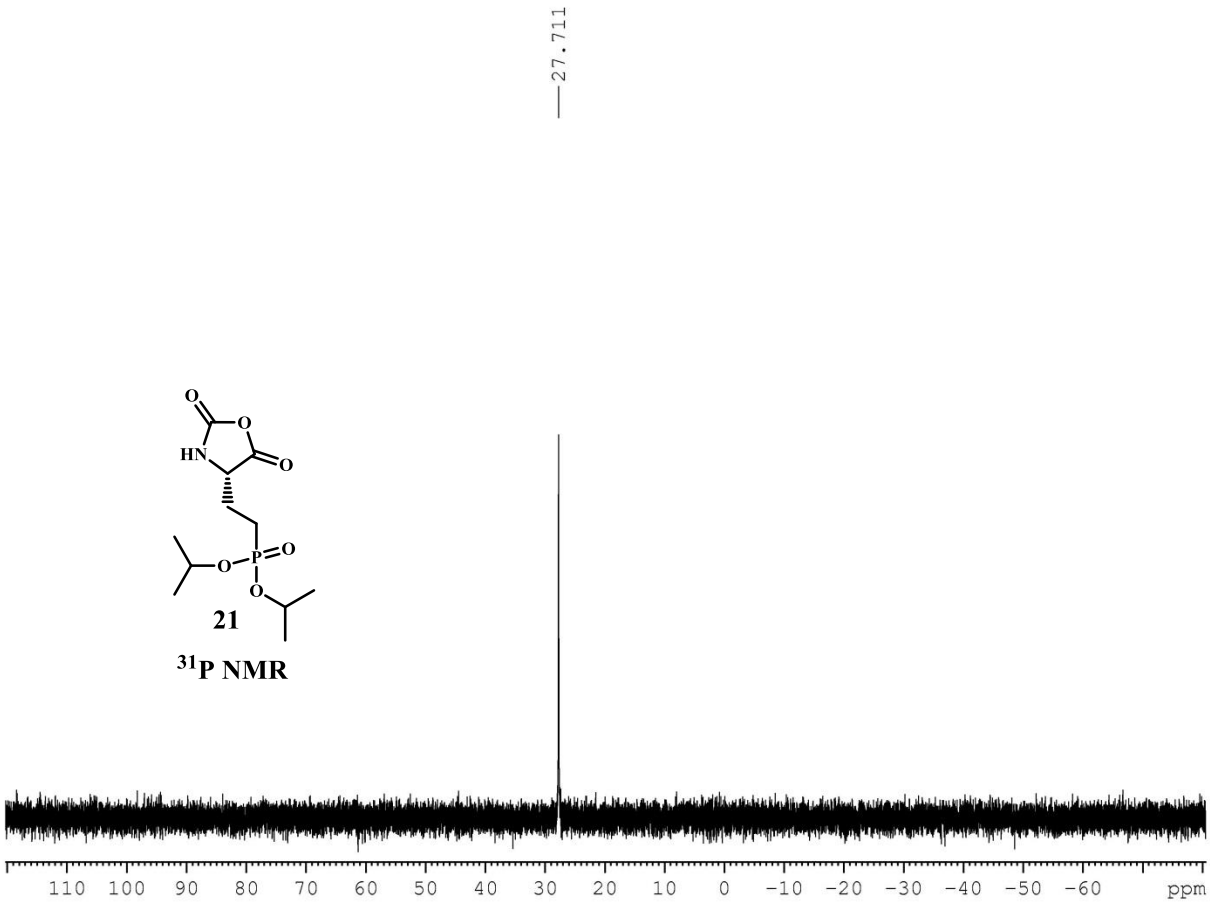
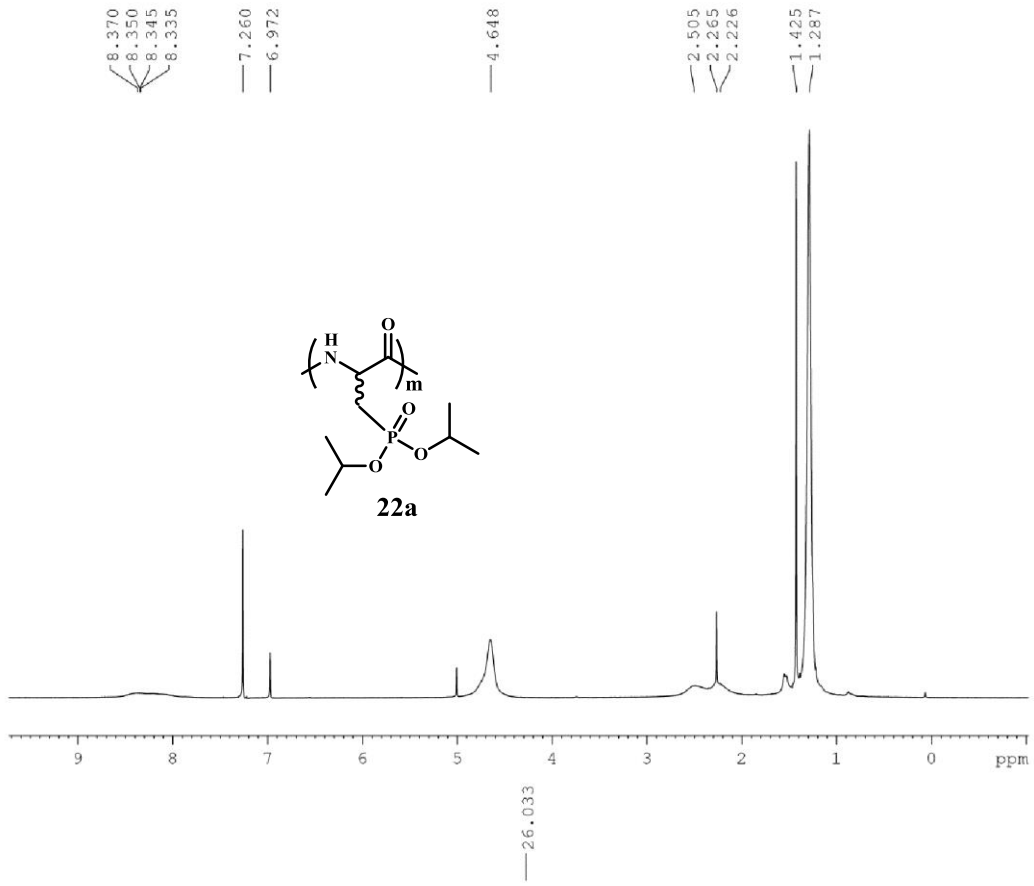
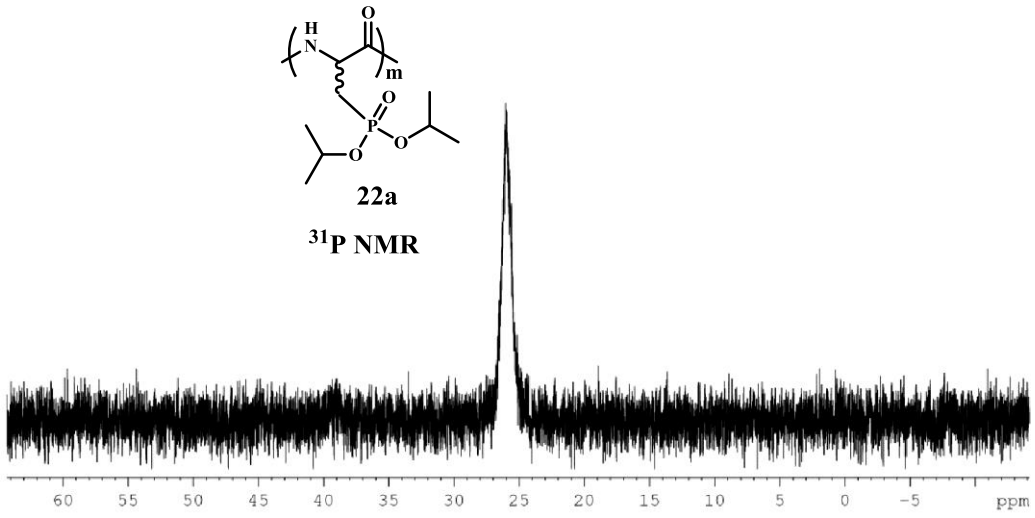
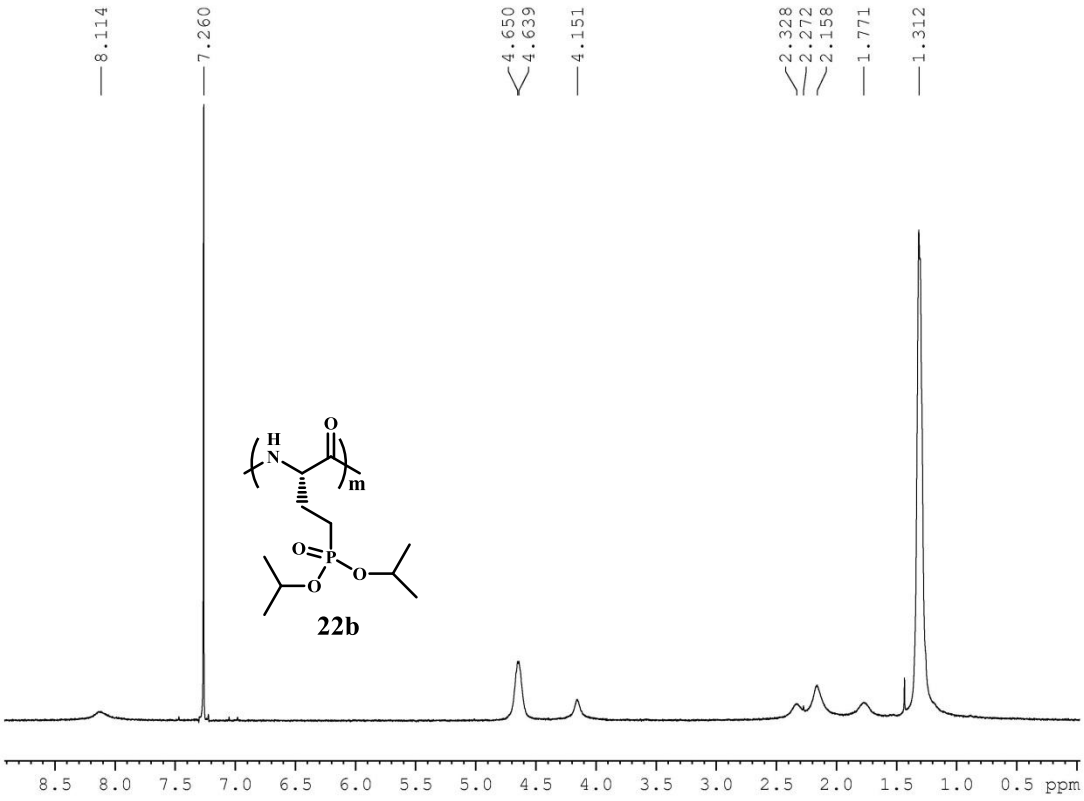
4.734
4.721
4.718
4.709
4.706
4.702
4.693
4.690
4.687
4.677
4.674
4.662
4.355
4.347
4.340
4.331
2.276
2.267
2.265
2.256
1.981
1.843
1.808
1.702
1.342
1.337
1.333
1.329
1.325
21
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

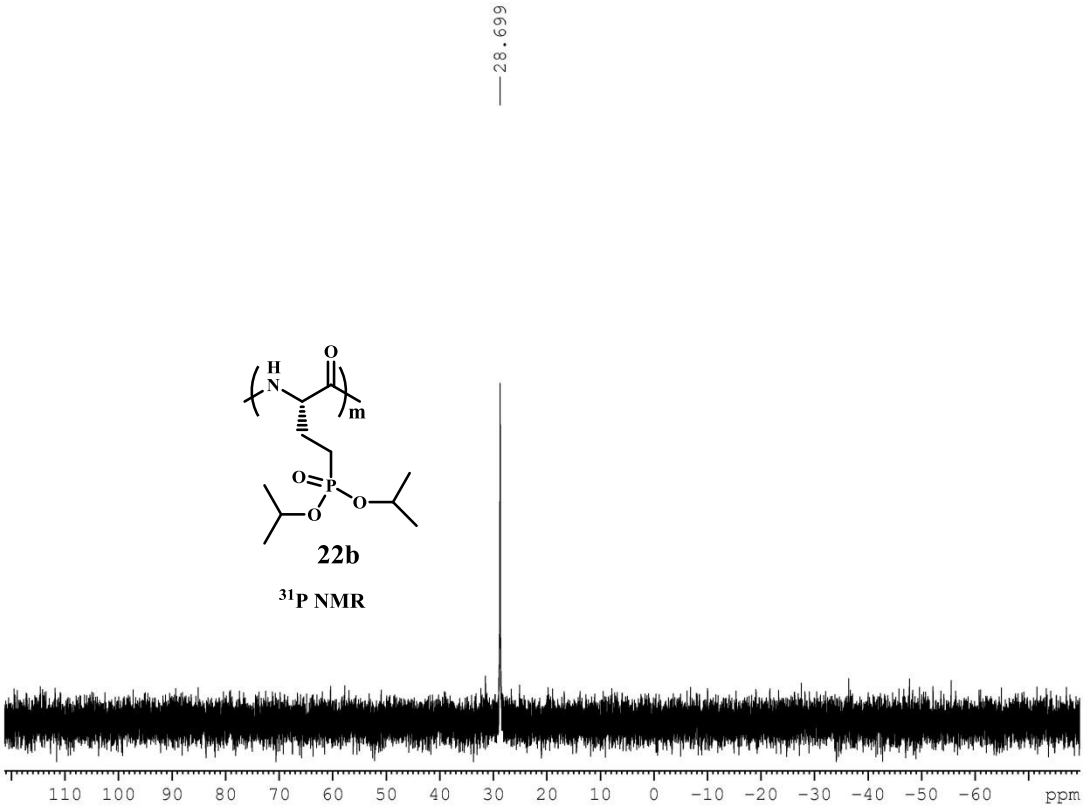

169.869
151.309
77.250
76.996
76.742
71.393
71.338
57.747
57.661
25.770
25.731
24.048
24.012
23.955
23.932
23.900
23.863
22.711
21
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

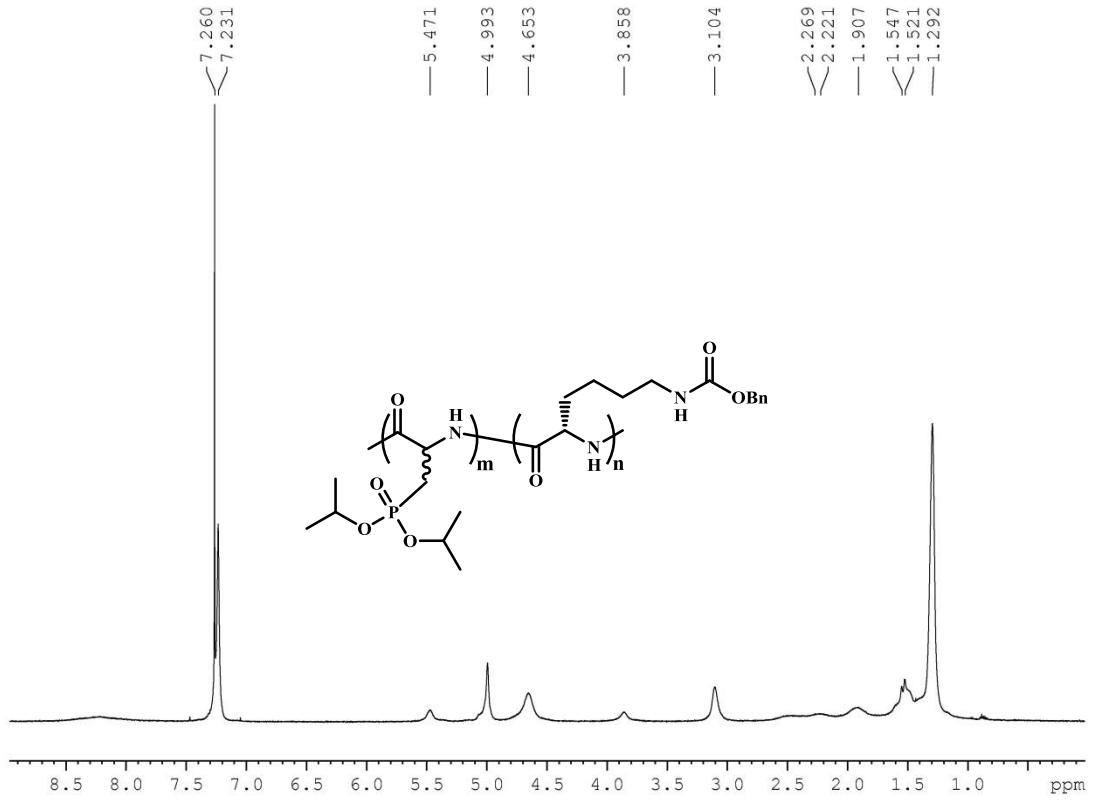

27.711
21
31P NMR
110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 ppm

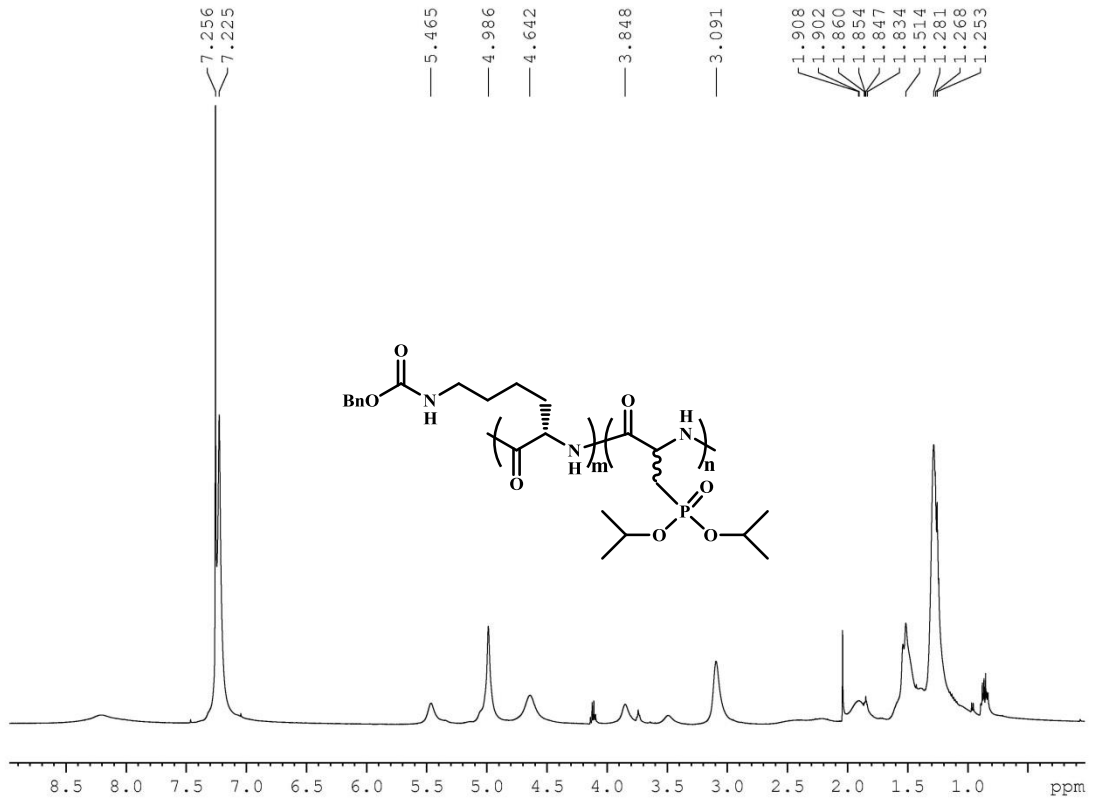

8.370
8.350
8.345
8.335
7.260
6.972
4.648
2.505
2.265
2.226
1.425
1.287
22a
9
8
7
6
5
4
3
2
1
0
ppm
26.033


22a
31P NMR
60
55
50
45
40
35
30
25
20
15
10
5
0
-5
ppm


8.114
7.260
4.650
4.639
4.151
2.328
2.272
2.158
1.771
1.312
22b
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm


28.699
22b
31P NMR
110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 ppm


7.260
7.231
5.471
4.993
4.653
3.858
3.104
2.269
2.221
1.907
1.547
1.521
1.292
OBn
m
n
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm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7.225
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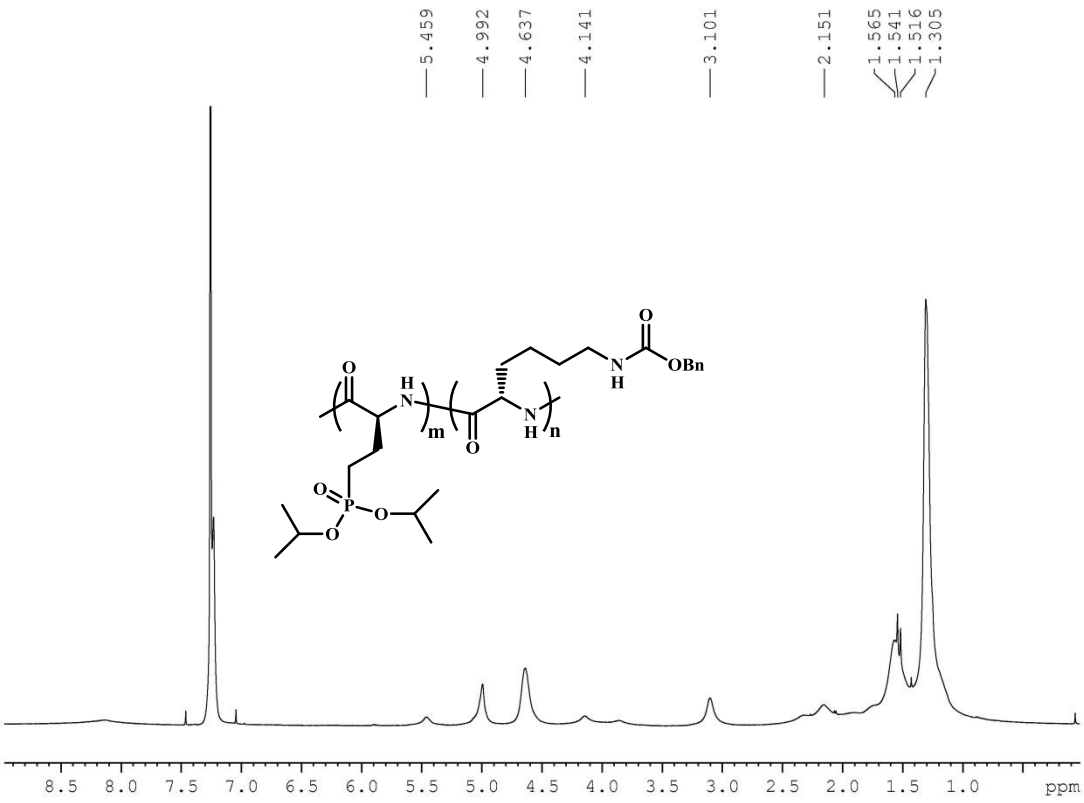
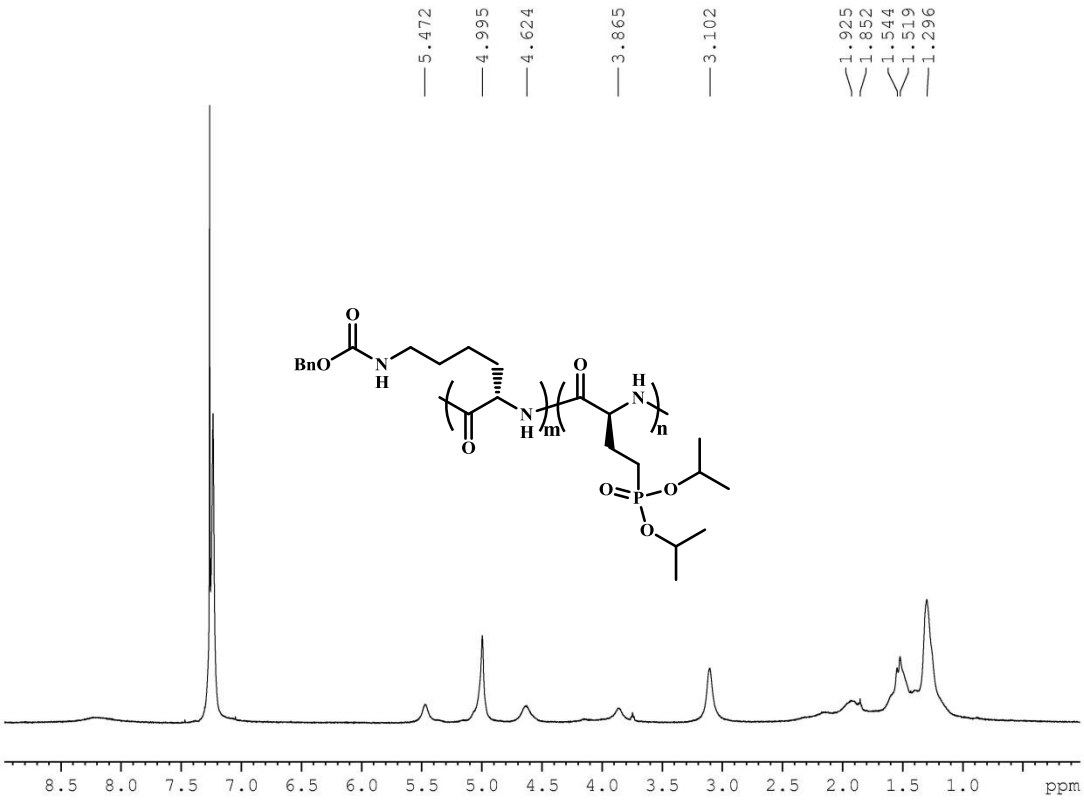
4.986
4.642
3.848
3.091
1.908
1.902
1.860
1.854
1.847
1.834
1.514
1.281
1.268
1.253
BnO
m
n
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm


5.459
4.992
4.637
4.141
3.101
2.151
1.565
1.541
1.516
1.305
OBn
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm


5.472
4.995
4.624
3.865
3.102
1.925
1.852
1.544
1.519
1.296
BnO
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm

CHAPTER THREE: Ca^{2+} Response of Block Copolypeptides Containing Analogs of Phosphoserine

3.1 Introduction

The capability to prepare inorganic materials of specific morphology, size, and polymorph has a number of valuable applications in fields of nanomaterials,[91] ceramics,[92] electronics,[93] regenerative medicine,[94] and many others.[95] Biological systems have been expertly forming minerals for millennia, mainly by using organic molecules as nucleators to form inorganic materials. Researchers have been mimicking nature by using synthetic additives, such as block copolypeptides to control the formation of inorganic materials. Due to our group's interest in the use of synthetically prepared polypeptide materials, we are naturally drawn to the prospect of using inorganic materials for biomedical applications. In the past we have shown that calcium carbonate, which typically crystallizes to form cubic calcite, can be made into calcium microspheres using block copolypeptide additives.[96] This work and previous research has shown that Ca^{2+} tends to complex with anionic polymers, can be based on a variety of monomers.[97-99] Poly(Pha), which was described in the previous chapter may serve as an excellent biomimetic and potentially biodegradable material in biomineralization research. Due to phosphonate groups' similar properties to phosphate moieties, phosphonates may serve to be selective Ca^{2+} binders and formation directors of highly ordered $CaCO_3$ minerals.

3.2 Poly(Pha) and its Interaction with Ca^{2+}

Qualitatively, we found that upon exposure to soluble Ca^{2+} salts such as $CaCl_2$ or $Ca(NO_3)_2$, poly(Pha) slowly forms insoluble polymer aggregates (Figure 3.1A). These aggregates only form only after addition of Ca^{2+} but not Na^{+} or Mg^{2+}. Furthermore, precipitation is accelerated at elevated temperature and decelerated at reduced temperature, a trend seen with calcium phosphate salts. These observations suggest that the effect stems from specific interactions with Ca^{2+}, and not due to salting out.

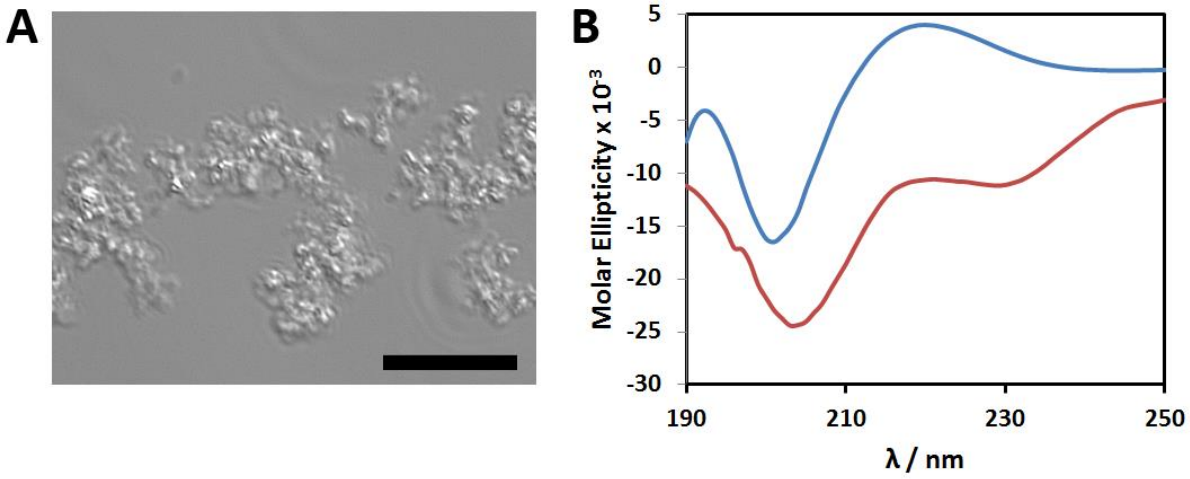


Figure 3.1. Poly(Pha) and its interactions with Ca^{2+}. (A) Optical microscopy image of poly(Pha) with 1.5 eq. of $CaCl_2$ or $Ca(NO_3)_2$, scale bar = 25 μm; (B) Circular dichroism spectrum of poly(Pha) in water at pH 8.0 (blue line) at pH 8.0 with 10 eq. per residue of $CaCl_2$ (red line).

Poly(Pha) adopts a disordered conformation at neutral and basic pH and an α-helical conformation in aqueous acidic solutions. We found that the addition of $CaCl_2$ to dilute solutions of polymer results in a conformation change to what appears to be a partially disordered helix (Figure 3.1B). When observed by FTIR, the polymer precipitate shows amide bands at 1620 and 1535 cm^{-1}, which suggests the presence of a beta sheet conformation (Figure 3.2). These

observations alone are not sufficient to fully characterize the nature of interactions between Ca^{2+} and poly(Pha); however the interactions appear to be specific to Ca^{2+}over other cations.

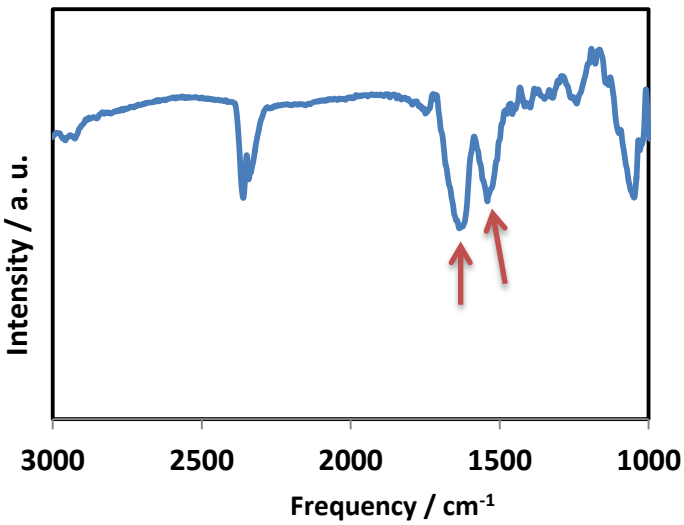


Figure 3.2. FTIR spectrum of precipitate formed by mixing of Ca^{2+} with poly(Pha) showing bands at 1620 and 1535 cm^{-1}.

3.3 Hydrophilic-Hydrophilic Block Copolypeptides that Direct the Formation of $CaCO_3$ Microspheres.

Based on previous work, we knew that doubly-hydrophilic diblock copolypeptides were capable of directing formation of $CaCO_3$ microspheres.[96] Following this design, we hypothesized that copolypeptides of **1** (Figure 3.3A) may serve a similar role. Poly(methionine sulfoxide) (poly(Met^O)) was chosen as a non-interacting water soluble segment. A poly(Met^O) control polymer, which was synthesized as described previously,[68] was dissolved in water and exposed to increasing concentrations of $CaCl_2$ and $Ca(NO_3)_2$. The polymer was found to stay in solution and did not show detectable aggregate formation by DLS.

In order to further explore the utility of poly(Pha), we prepared a series Met^O-Pha diblock copolypeptides using $Co(PMe_3)_4$ initiated polymerization in THF. Segments of $poly(Met)_{50}$ were

synthesized first, followed by adding varying amounts of Pha NCA to the living chain ends. Pha segments of the resulting hydrophobic-hydrophobic $Met_{50}Pha_n$ diblocks were deprotected using iodotrimethylsilane (TMSI). Met segments were then oxidized to Met^O using a dilute solutions of H_2O_2 at 37°C. The order of deprotection and oxidation is important, as TMSI is known to reduce sulfoxides to thioethers.[100,101] Methionine segment lengths were determined using end-group analysis by NMR (active chains were end-capped with isocyanate-terminated PEG; M_n = 1000),[87] and were compared to the Pha segment by ^1H NMR to obtain total diblock lengths (Figures 3.3 and 3.4). GPC analysis could not be carried out due to the insolubility of poly(methionine) in DMF.

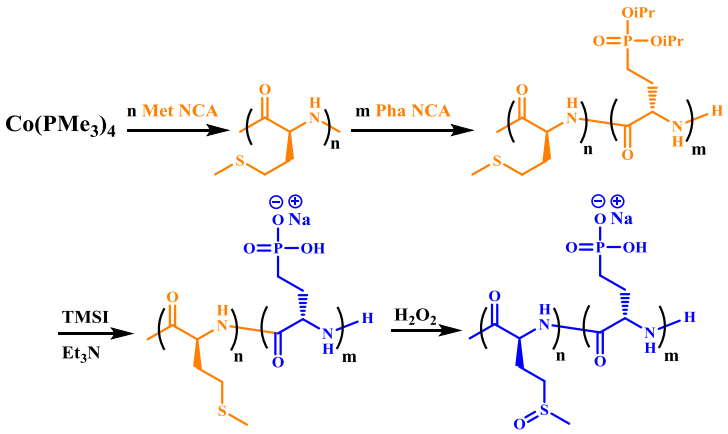


Figure 3.3. Synthesis of diblock copolypeptides.

First Monomer[a]	Second Monomer[a]	First Segment[b] M_n	First Segment[b] D_p[d]	Diblock Copolymer[c] M_n	Diblock Copolymer[c] D_p[d]	Yield (%)[e]
22 Met NCA	4 Pha NCA	6 600	50	7800	55	92
22 Met NCA	7 Pha NCA	6 600	50	9800	63	90
22 Met NCA	11 Pha NCA	6 600	50	11500	70	91
22 Met NCA	15 Pha NCA	6 600	50	13800	79	92

Figure 3.4. Synthesis of diblock copolypeptides. [a] Number of equivalents of monomer per $Co(PMe_3)_4$. [b] Molecular weight of first segments determined for PEG-NCO (1 kDa) end-capped samples using ^1H NMR. [c] Molecular weight of diblock copolymers determined for using ^1H NMR. [d] DP = degree of polymerization. [e] Total isolated yield of purified hydrophobic-hydrophobic diblock copolypeptide.

The deprotected and oxidized diblock copolypeptides were examined for $CaCO_3$ microsphere formation ability using a modified procedure of our previously published work.[96] Polymers were dissolved in 0.5 M Na_2CO_3 that was adjusted to pH 10 using 1.0 M HCl. A solution of 0.5 M $CaCl_2$ was quickly added as the mixture was vortexed; the vortexing was continued for 2 more minutes following the addition. $CaCO_3$ precipitate formed slowly over 24 hours. Following the incubation, the excess salts were washed away with water and the insoluble powder was collected by centrifugation. All of the $Met^{O}_{n}Pha_{m}$ block copolypeptides formed $CaCO_3$ microspheres, with $Met_{50}Pha_{13}$ being the optimal composition for consistent sphere formation.

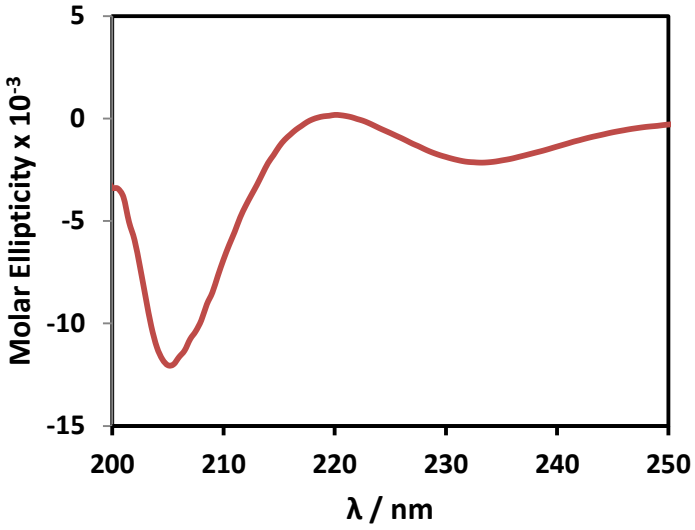


Figure 3.5. Circular dichroism spectrum of **1** in water at pH 10 (1 mg/ml).

A larger batch of diblock copolypeptide **1** ($Met^{O}_{65}Pha_{20}$) of the same relative composition was then prepared for further studies. The polymer was highly soluble in water over a range of pH (2-12) and possessed a disordered conformation, as expected (Figure 3.5). Microsphere growth using polypeptide **1** was successfully accomplished using the aforementioned method (Figure 3.6).

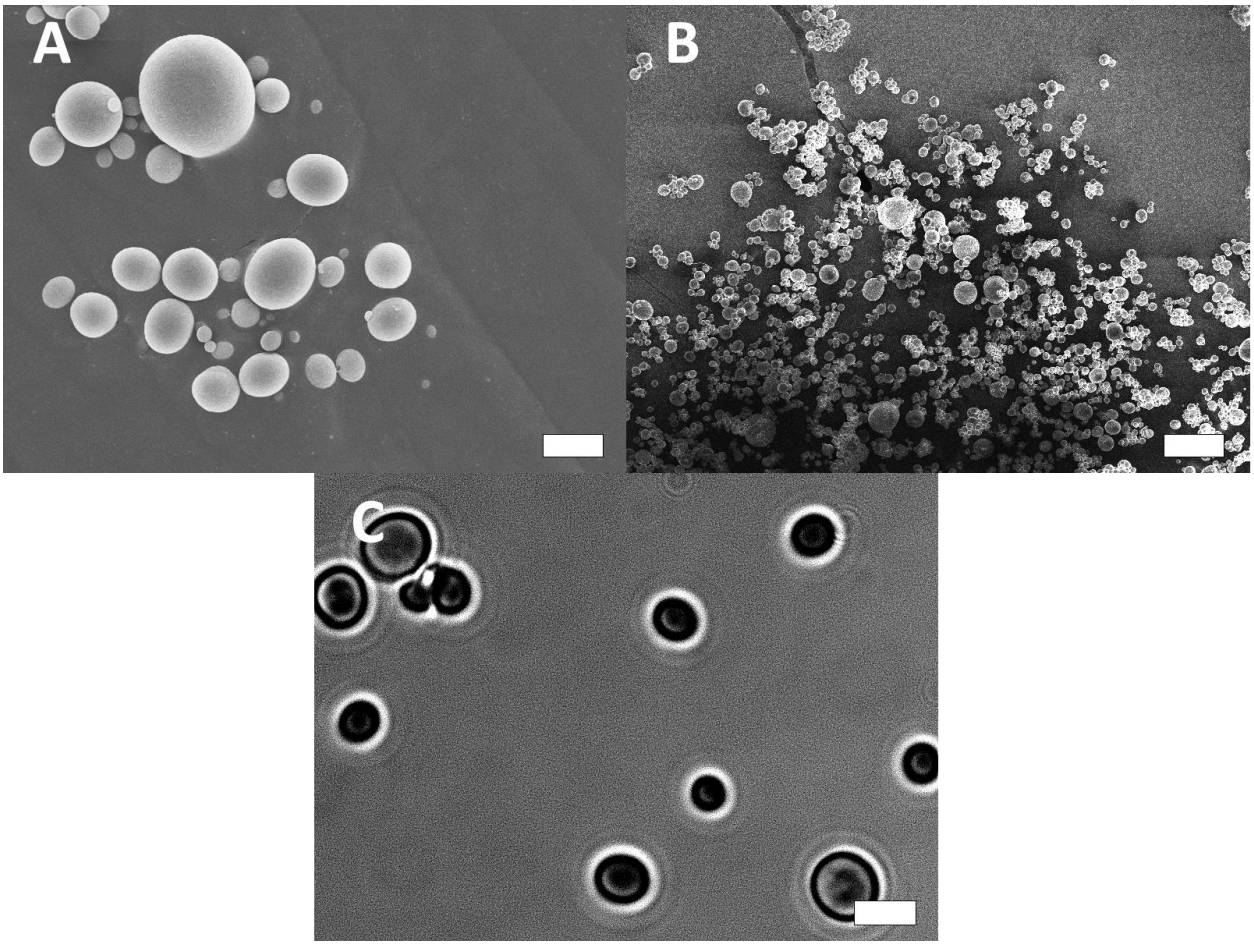


Figure 3.6. SEM micrographs of $CaCO_3$ spheres (A, scale bar = 5 μm, B, scale bar=22 μm) and light microscopy images of $CaCO_3$ spheres (C, scale bar = 5 μm). 0.1 mg/ml of polymer.

Control experiments with no polymer or poly(Met^O) yielded cubic crystals of $CaCO_3$ while controls containing poly(Pha) gave irregular fused spheres of $CaCO_3$ (Figure 3.8). These results confirmed that diblock copolymer architecture was essential for selective formation of $CaCO_3$ spheres over other morphologies.

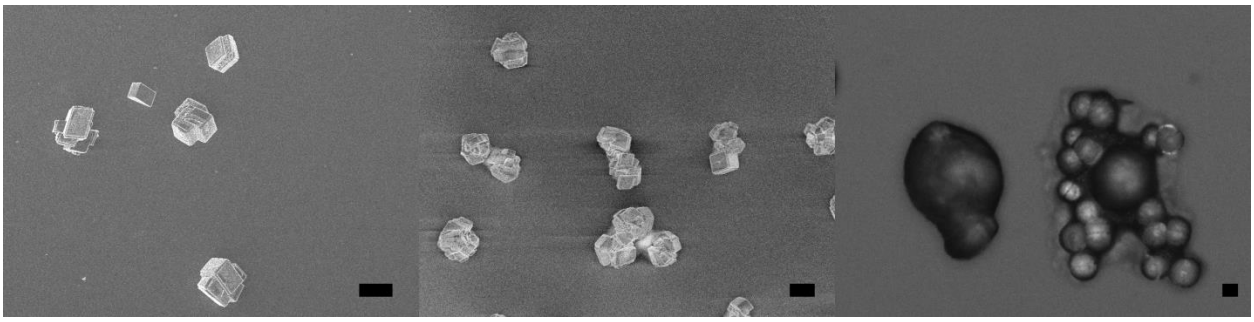


Figure 3.7. Images of calcium carbonate growth in control experiments. Poly(Met^{O}) (0.10 mg/ml, left, SEM), no polymer (middle, SEM), poly-Pha (right, light microsopy) all scale bars = 10 μm

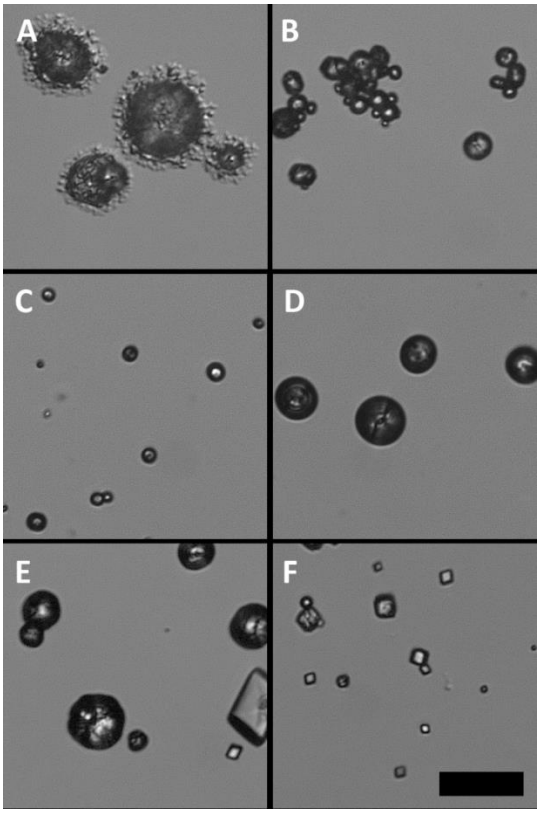


Figure 3.8. Light microscopy images of sphere morphology as a function of polymer **1** concentration: (A) 0.5 mg/ml; (B) 0.25 mg/ml; (C) 0.1 mg/ml; (D) 0.005 mg/ml; (E) 0.001 mg/ml; (F) 0.0001 mg/ml; Scale bar = 25μm.

Changes in microsphere size and shape were observed with varying concentrations of copolymer **1**. Morphologies appeared to have an inverse relationship to the amount of polymer

(Figure 3.9). These observations are in agreement with previous research, and can be rationalized in a similar manner.[96] The phosphonate segment of copolymer **1** interacts with Ca^{2+} in solution, causing a local supersaturation. The non-Ca^{2+} binding Met^{O} segment acts as a steric stabilizer by limiting aggregation of poly(Pha) segments, which allows for the formation of $CaCO_3$ spheres. When a higher concentration of **1** is present, a larger number of spheres are nucleated, resulting in a smaller average diameter (Figure 3.9 A – C). A lower concentration results in larger spheres (Figure 3.9 D and E). Interestingly, when a very high concentration of **1** is used, small spheres group together to form large compound spheres, which appear to be coated with smaller spheres on their surface (Figure 3.9 A). At increasingly low concentrations, mixtures of spheres large and crystals followed by observation of only large crystals (Figure 3.7 E and F).

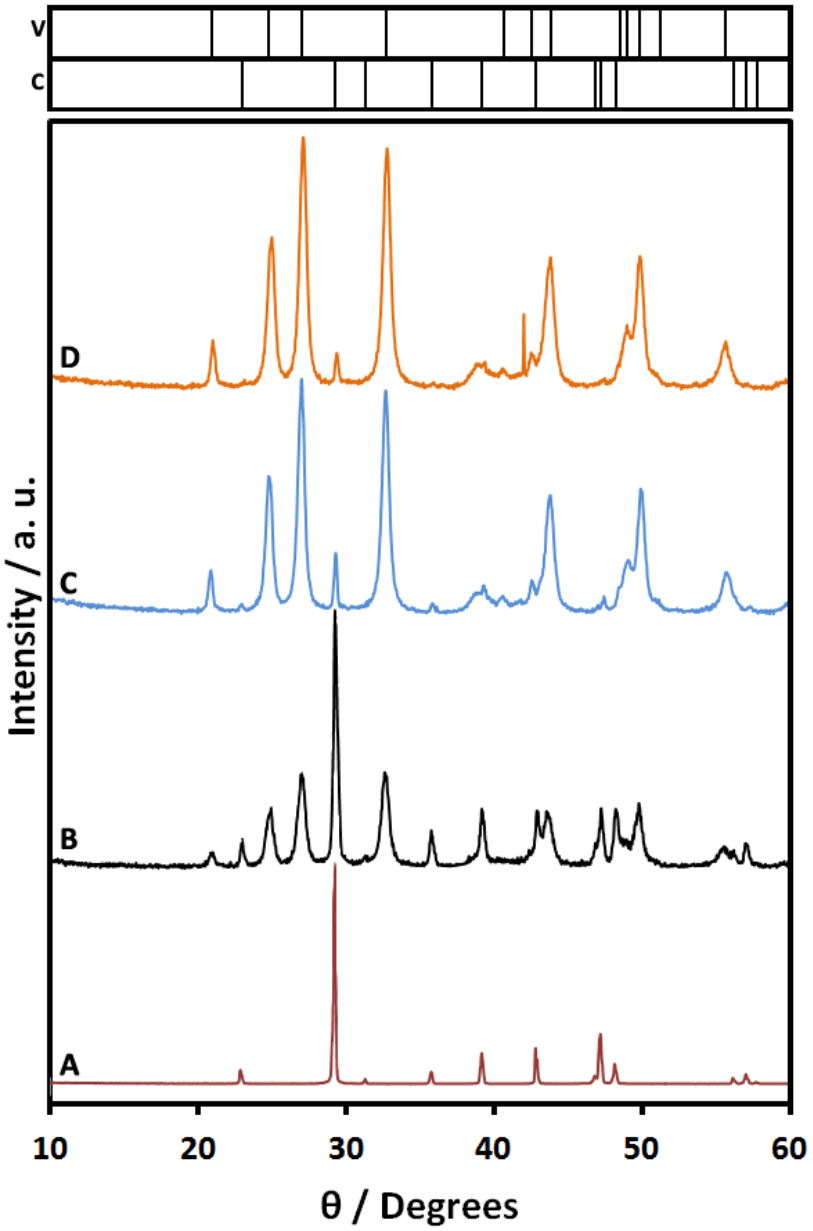


Figure 3.9. X-ray diffraction pattern of control (red) and X-ray patterns with different addition rates of $CaCl_2$ to solutions of **1** (A → no $CaCl_2$ control, B→ one portion, C → 1 minute, D → 2 minutes).

Powder X-ray diffraction experiments show that spheres are comprised of a mixture of calcite and vaterite forms of $CaCO_3$. Preparation conditions can be slightly altered to form mainly vaterite, simply by slowing down addition of $CaCl_2$ into solutions of **1**. When slowed from single addition (Figure 3.10B) to addition over 2 minutes (Figure 3.10D), an almost complete conversion to vaterite is observed.

Finally, **1** was extracted from $CaCO_3$ microspheres to confirm the polymer's presence within the spheres. The spheres were dissolved in 1.2 M HCl and the resulting solution was dialyzed against deionized water for 3 days with twice daily water changes. Following lyophilization, **1** was obtained from the sample in >90% recovery.

3.4 Conclusion

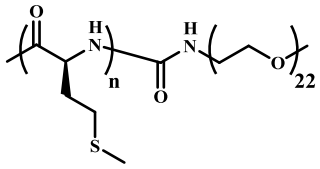
Poly(Pha) is the second ever phosphonate containing polypeptide to have been prepared using polymerization. This chapter describes the first synthesis of water soluble diblock copolypeptides containing poly(Pha). These diblocks are able to bind Ca^{2+}, which to the best of our knowledge, has not been observed with any phosphonate-containing polymer. We believe that the interesting properties of poly(Pha) combined with its synthetic versatility will allow poly(Pha) to gain traction in numerous exciting applications.

3.5 Experimental

3.5.1 Materials and Methods

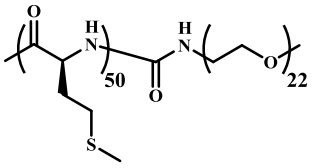
Unless otherwise stated, reactions were carried out in oven-dried glassware under an atmosphere of nitrogen using anhydrous solvents. THF, hexanes, DCM, and toluene were purified by passage through activated alumina-packed columns under nitrogen. All reagents were ordered from Aldrich, Spectrum, Alfa Aesar, Bachem, or CombiBlocks, and were used as received unless otherwise stated. ^{1}H NMR spectra were recorded at 500MHz on Bruker instruments; ^{13}C NMR spectra were recorded on 125MHz Bruker instruments with the solvent peak as internal reference. All Fourier Transform Infrared (FTIR) samples were prepared as thin films on NaCl plates and spectra were recorded on a Perkin Elmer RX1 FTIR spectrometer and are reported in terms of frequency of absorption (cm^{-1}). SEM samples were plated with ~10 nm of gold using a Hummer 6.2 sputtering system. SEM images were recorded on a JEOL SM-71010 scanning electron microscope. The samples were imaged using a Zeiss Axiovert 200 DIC/Fluorescence Inverted Optical Microscope.

3.5.2 Experimental Procedures

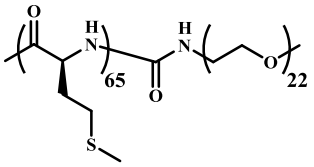


Synthesis of Methionine-*block*-PEG1000. The polymerization of methionine NCA was performed in a nitrogen filled glove box using anhydrous, nitrogen purged solvents. To 200 µl of a 50 mg/ml solution of *L*-methionine N-carboxyanhydride (M NCA) in THF was added a desired amount of $Co(PMe_3)_4$ in THF (10 mg/ml). The reaction was allowed to polymerize for 2 hours, at which point the NCA was consumed as confirmed by FTIR. The reaction was then treated

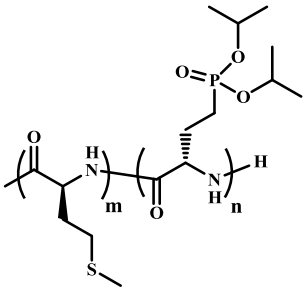
with an excess of polyethylene glycol isocyanate (PEG-NCO, MW = 1000 Da, 3 equiv per $Co(PMe_3)_4$). The resulting solution was allowed to react for 3 hours. The reaction was then brought outside of the glove box and was precipitated into 1 ml of 1.0M HCl; the pellet was collected by centrifugation and was washed twice more with 1ml of diH_2O each time. The residual water was then removed by lyophilization and the polymer was analyzed by ^{1}H NMR.



^{1}H NMR (500 MHz, *d*TFA) δ 4.88 (47H, br s), 3.93 (90H, br s, PEG signal), 2.71 (100H, br s, signal used for length determination), 2.19 (245H, overlapping peaks).

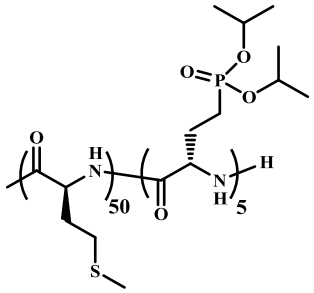


^{1}H NMR (500 MHz, *d*TFA) δ 4.78 (63H, br s), 3.83 (90H, br s, PEG signal), 2.60 (130H, br s, signal used for length determination), 1.91-2.17 (338H, overlapping peaks).

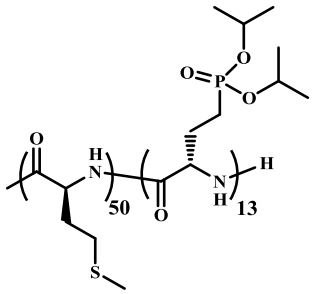


General procedure for the synthesis of Met-Pha diblock copolypeptides. The copolymerizations were performed in a nitrogen filled glove box using anhydrous, nitrogen purged solvents. To a 50 mg/ml solution of M NCA in THF was added a solution with a desired amount of $Co(PMe_3)_4$ in THF (10 mg/ml). The reaction was allowed to polymerize for 2 hours,

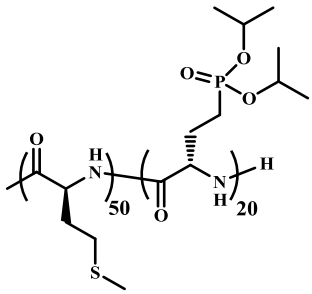
at which point the NCA was consumed as confirmed by FTIR. A desired amount of a 50 mg/ml solution of Pha NCA was then added and the reaction was left to polymerize overnight. The next day, the consumption of the second NCA was confirmed by IR and the polymer was isolated by precipitation into 1.0 M HCl (1 ml per 10mg of total NCA), followed by resuspension in water (1 ml per 10 mg of total NCAs) and centrifugation to collect the pellet. The residual water was removed by lyophilization to obtain the polymer as a white, stringy solid.



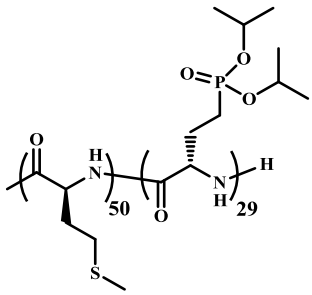
^{1}H NMR (500 MHz, *d*TFA) δ4.76-5.03 (67H, overlapping peaks), 2.77 (100H, br s, Met Methyl, used as a NMR reference), 1.97-2.43 (273H, overlapping peaks), 1.41 (58H, Pha iPr, used to calculate length of second block). FTIR (THF): 1644, 1550 cm^{-1}. 92% yield.



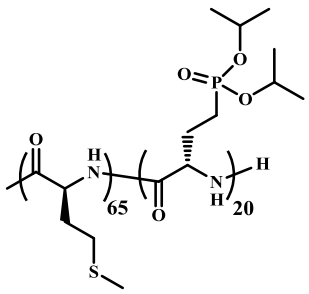
^{1}H NMR (500 MHz, *d*TFA) δ4.76-5.03 (91H, overlapping peaks), 1.89-3.13 (434H, overlapping peaks), 1.42 (184H, br s). FTIR (THF): 1644, 1550 cm^{-1}. 90% yield.



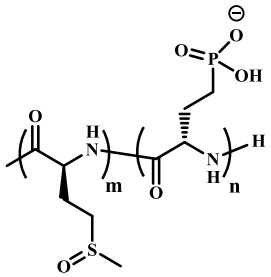
^{1}H NMR (500 MHz, *d*TFA) δ4.76-5.03 (117H, overlapping peaks), 1.89-3.13 (451H, overlapping peaks), 1.42 (281H, br s). FTIR (THF): 1644, 1550 cm^{-1}. 91% yield.



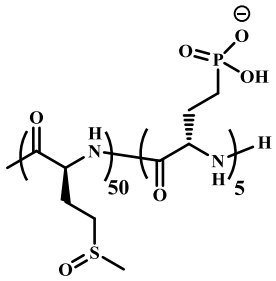
^{1}H NMR (500 MHz, *d*TFA) δ4.76-5.03 (143H, overlapping peaks), 1.89-3.13 (470H, overlapping peaks), 1.42 (415H, br s). FTIR (THF): 1644, 1550 cm^{-1}. 92% yield.



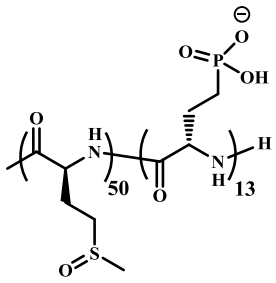
^{1}H NMR (500 MHz, *d*TFA) δ 4.00-5.04 (112H, overlapping peaks), 1.89-3.13 (537H, overlapping peaks), 1.42 (228H, br s). FTIR (THF): 1644, 1550 cm^{-1}. 96 % yield.



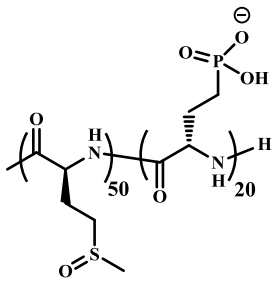
Synthesis of deprotected Met$^{(O)}$-Pha diblock copolypeptides. The Met-Pha diblock copolypeptide was dissolved in dry dichloromethane (20-30 mg/ml). The solution was treated with iodotrimethylsilane (10 eq. per Pha residue) and triethylamine (8 eq. per Pha residue). The reaction was sealed under nitrogen and stirred at 50 ^{o}C overnight. The next day, the solution was allowed to cool to room temperature and was treated with 1ml of isopropanol. The resulting solution was then precipitated into hexane and the solid that formed was collected by centrifugation. The pellet was then stripped of volatiles under high vacuum and the residue was suspended in 0.1 M HCl and then collected by centrifugation once again. The pellet was then resuspended in DI water (~1 ml per 20 mg of original polymer). This suspension was treated with 30% hydrogen peroxide (3µl per 1 mg or original polymer) and was stirred at 38 ^{o}C for 2 hours. After the stirring was complete, the resulting clear solution was cooled to 0 ^{o}C and treated with concentrated sodium thiosulfate to quench the excess hydrogen peroxide. The solution was then transferred to a 2000 MWCO dialysis bag and was dialyzed against 0.1 M sodium sulfite followed by 0.1 M NaCl, followed by water for 3 more days with twice daily water changes. Once the dialysis was complete, the water was removed by lyophilization to afford the product as a fluffy, stringy solid.



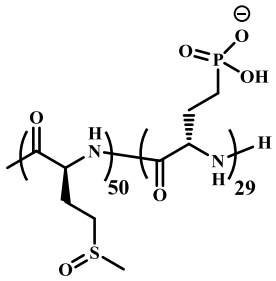
^{1}H NMR (300 MHz, D_2O) δ 4.51 (54H, br s), 2.86-3.10 (99H, br s), 2.75 (150H, br s), 2.26 (111H, br s), 1.55-2.04 (32H, br s). 96% yield.



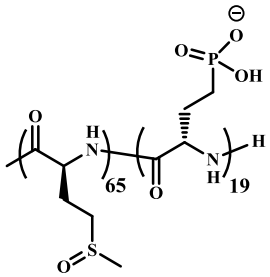
^{1}H NMR (300 MHz, D_2O) δ 4.51 (69H, br s), 2.86-3.10 (109H, br s), 2.75 (150H), 2.26 (125H, br s), 1.55-2.04 (41H, br s). 99% yield.



^{1}H NMR (300 MHz, D_2O) δ 4.51 (66H, br s), 2.86-3.10 (96H, br s), 2.75 (150H), 2.26 (111H, br s), 1.55-2.04 (80H, br s). 97% yield.



^{1}H NMR (300 MHz, D_2O) δ 4.51 (79H, br s), 2.86-3.10 (103H, br s), 2.75 (150H), 2.26 (120H, br s), 1.55-2.04 (115H, br s). 97% yield.



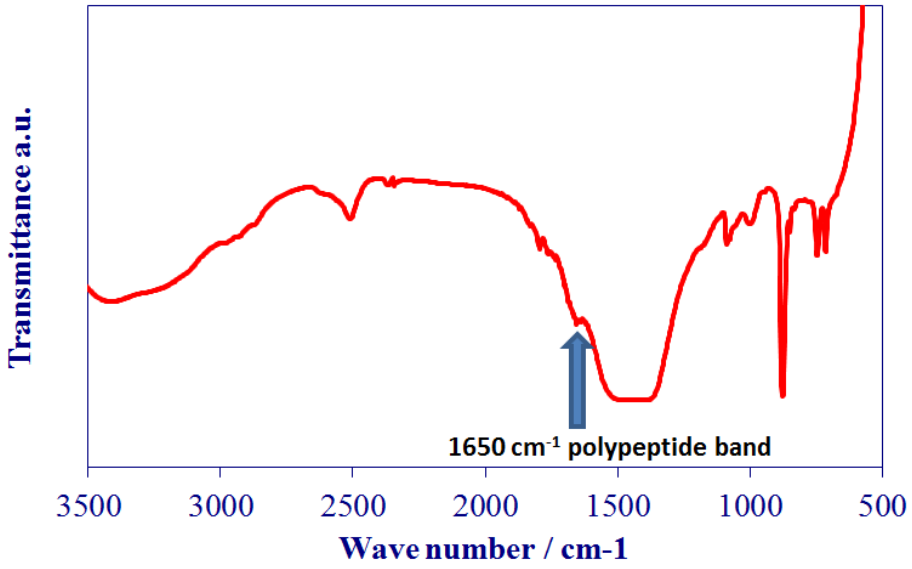
^{1}H NMR (500 MHz, dTFA) δ 4.87 (94H, br s), 2.19-3.38 (560H, overlapping signals), 1.40 (39H, br s); ^{1}H NMR (500 MHz, D_2O) δ 4.49 (66H, br s), 2.90-3.05 (137H, br s), 2.73 (195H, br s), 2.27 (70H, br s), 2.19 (64H, br s), 1.26 (82H, br s), 0.88 (35H, br s). 89% yield.

3.5.3 Preparation and Characterization of Spheres

Preparation of $CaCO_3$ microspheres. A desired concentration of copolymer was dissolved in 10 ml of Millipore water in a 50 ml conical centrifuge tube. 160 µl of 0.5 M Na_2CO_3 were then added and the solution was adjusted to pH 10 using 1.2 M HCl. The tube was then vortexed and 160 µl of 0.5 M $CaCl_2$ were added in a single portion. The solution was vortexed for another 2 minutes and was left undisturbed for 24 hours. The precipitate was then collected by centrifugation, was washed twice with Millipore water and was then freeze dried.

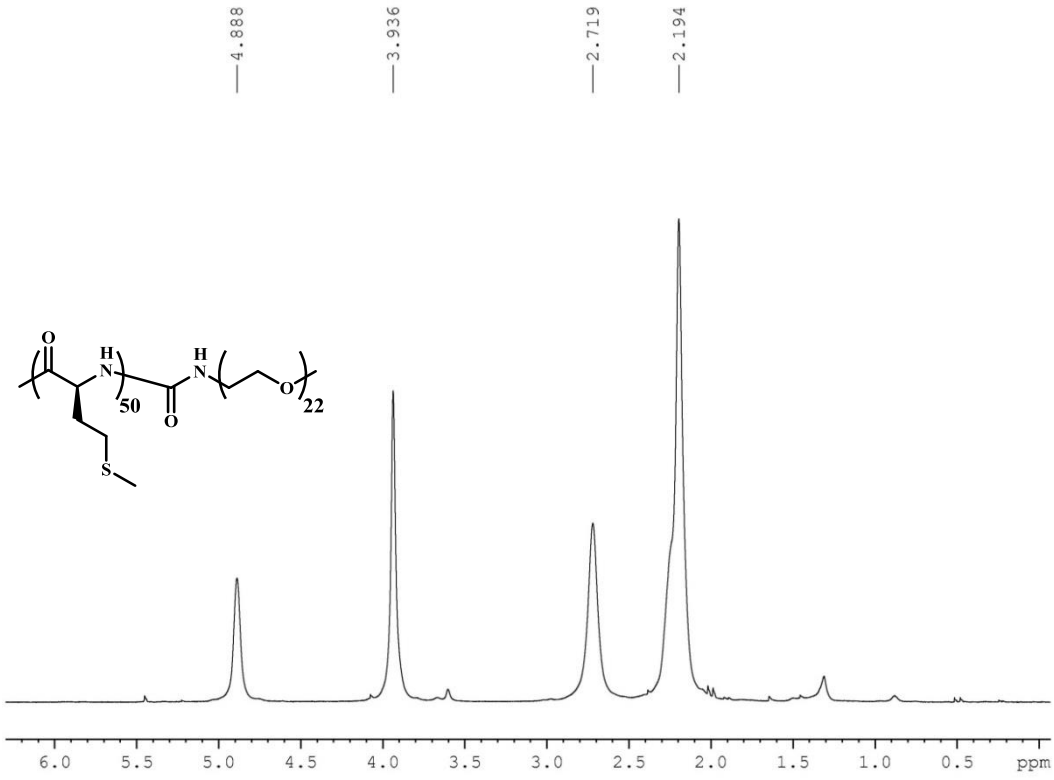
Light microscopy of microspheres. Spheres were imaged either wet or dry. For dry imaging, a small amount of powder was transferred onto a microscope slide. Powder was spread across the slide using a piece of filter paper and was imaged without any special precautions. For higher magnification, a drop of water was added onto the slide and the resulting suspension was covered with a cover slip. A drop of oil was then placed on top of the cover slip to obtain images using the oil immersion lens.

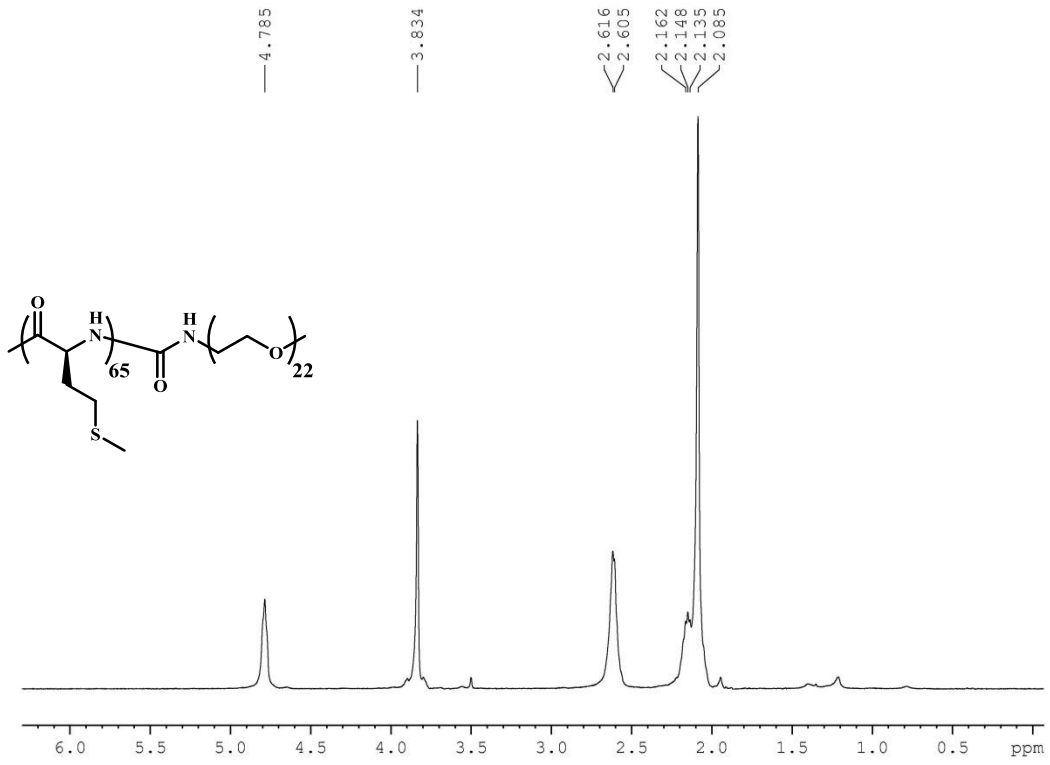
FTIR of spheres. The spheres were ground using a mortar and pestle in a 1:20 ratio with KBr. The pellet was examined by FTIR, 256 scans were collected.

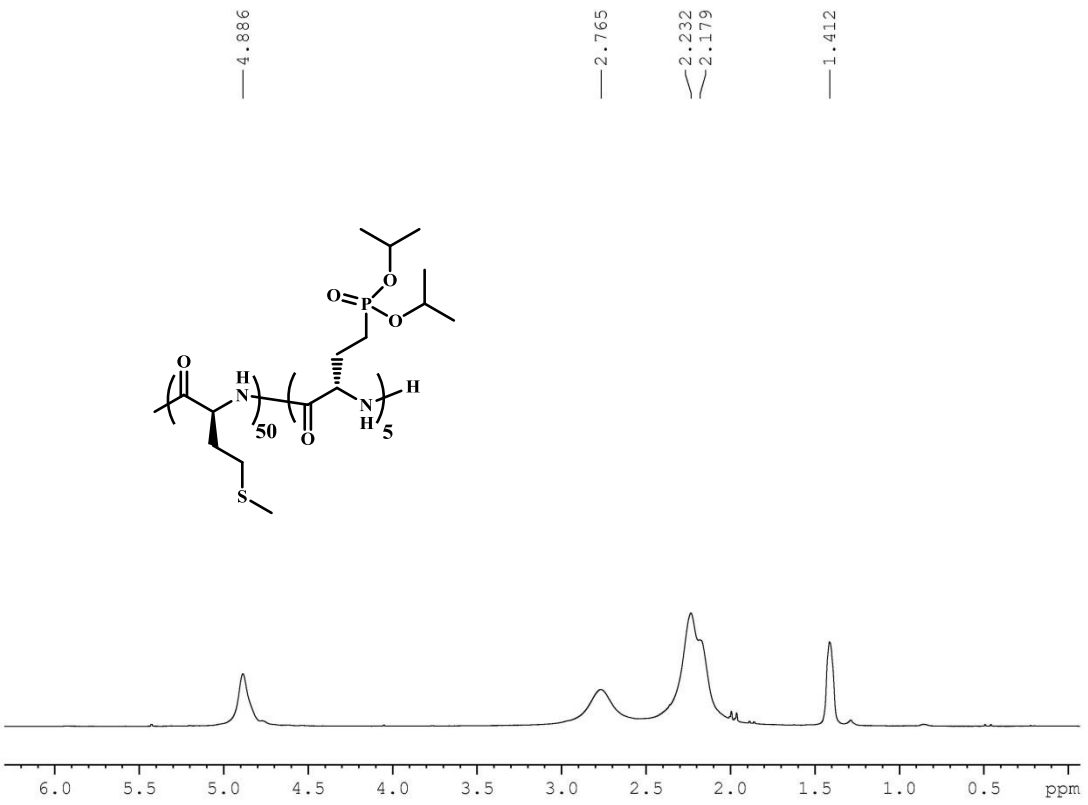


Extraction of copolymer from spheres. $CaCO_3$ polymer spheres were dissolved in 1.0 M HCl, their dissolution was nearly instant. The solution was transferred into a 2000 MWCO dialysis membrane and was dialyzed against deionized water for 3 days with twice daily water changes. The sample was then freeze dried and the resulting stringy solid was analyzed by NMR. Greater than 95% recovery of copolymer from spheres was attained. The extracted copolymer samples were identical by NMR to the copolymers used in sphere preparation.

3.5.4 Spectral Data



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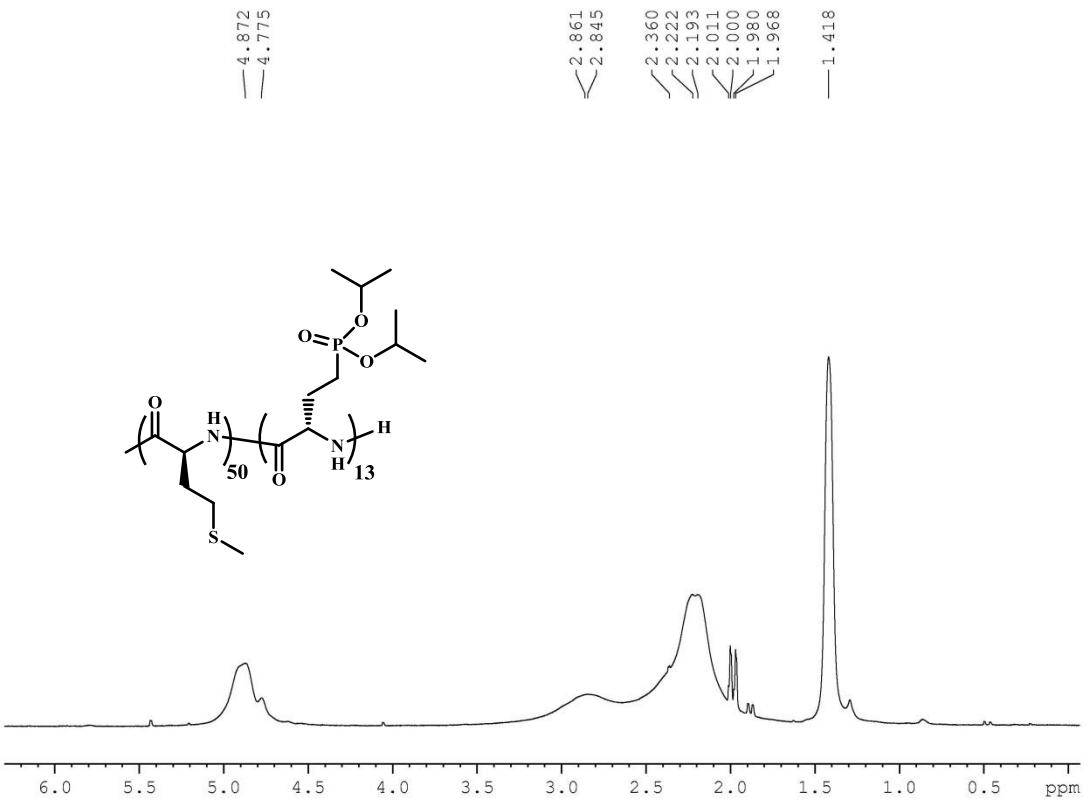
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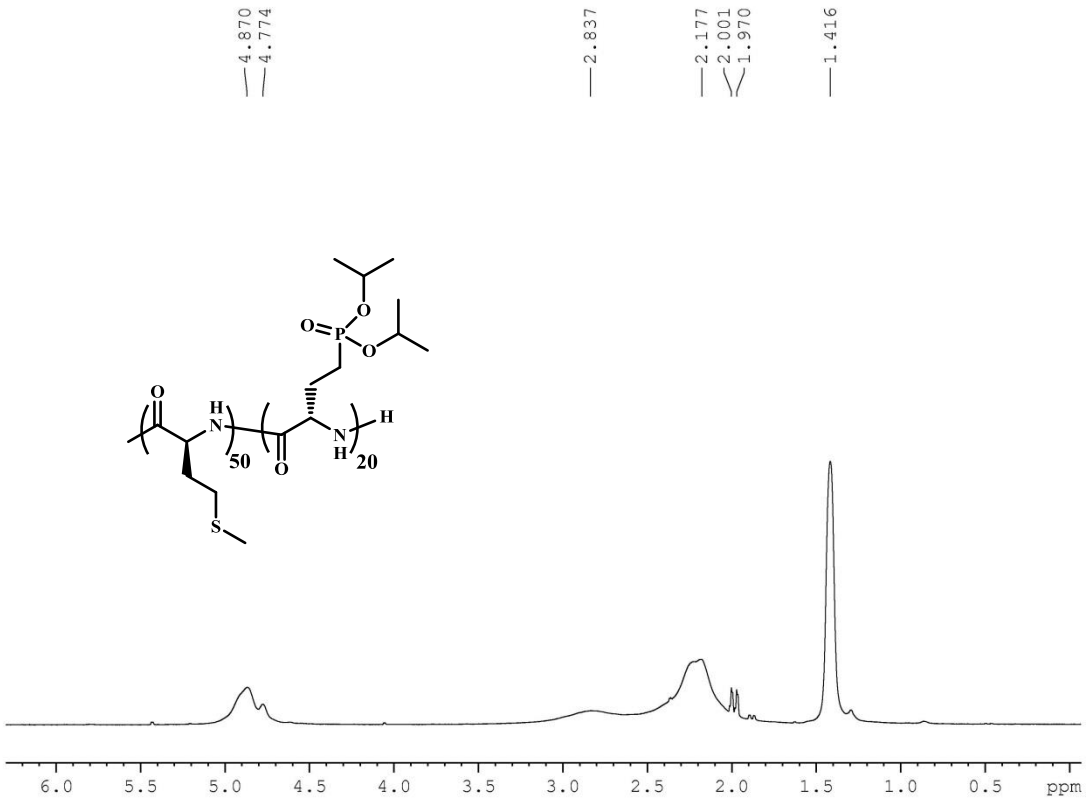
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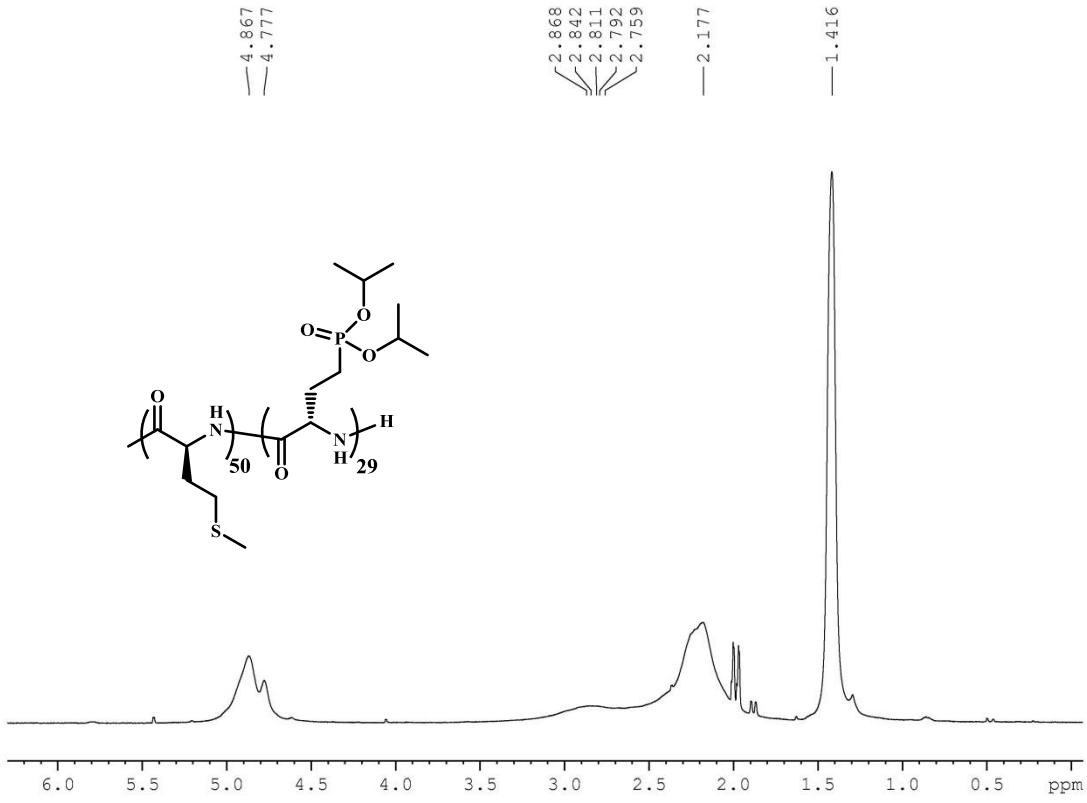
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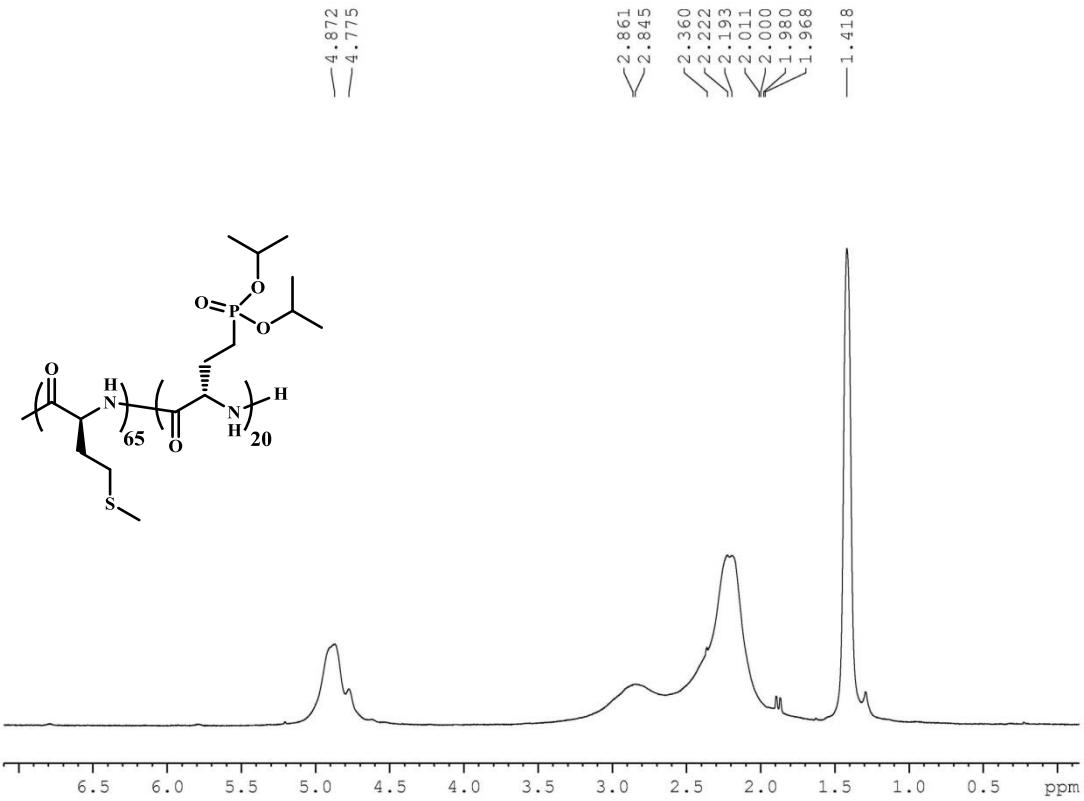
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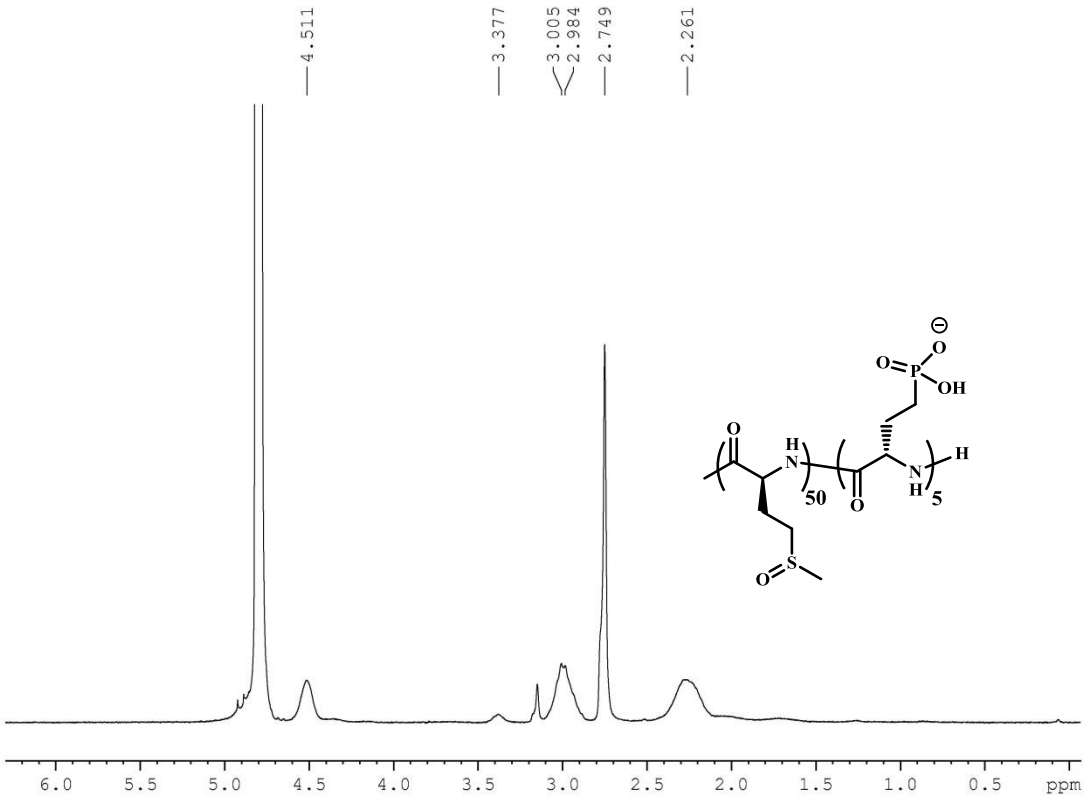
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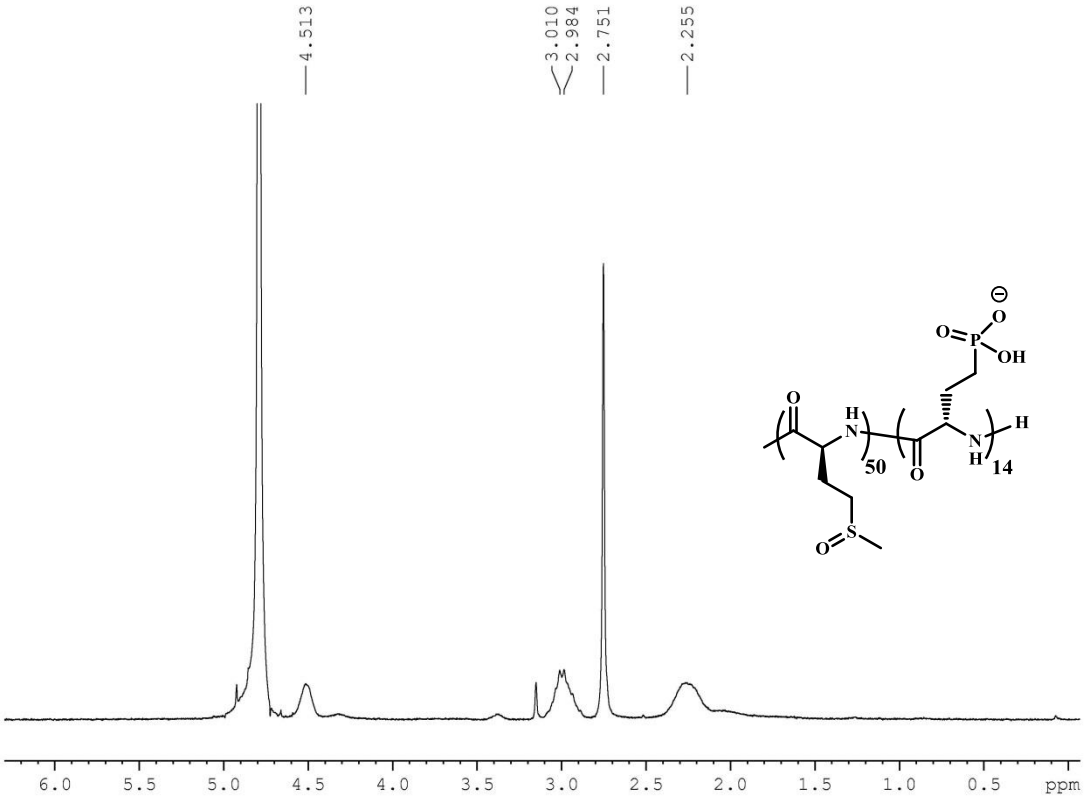
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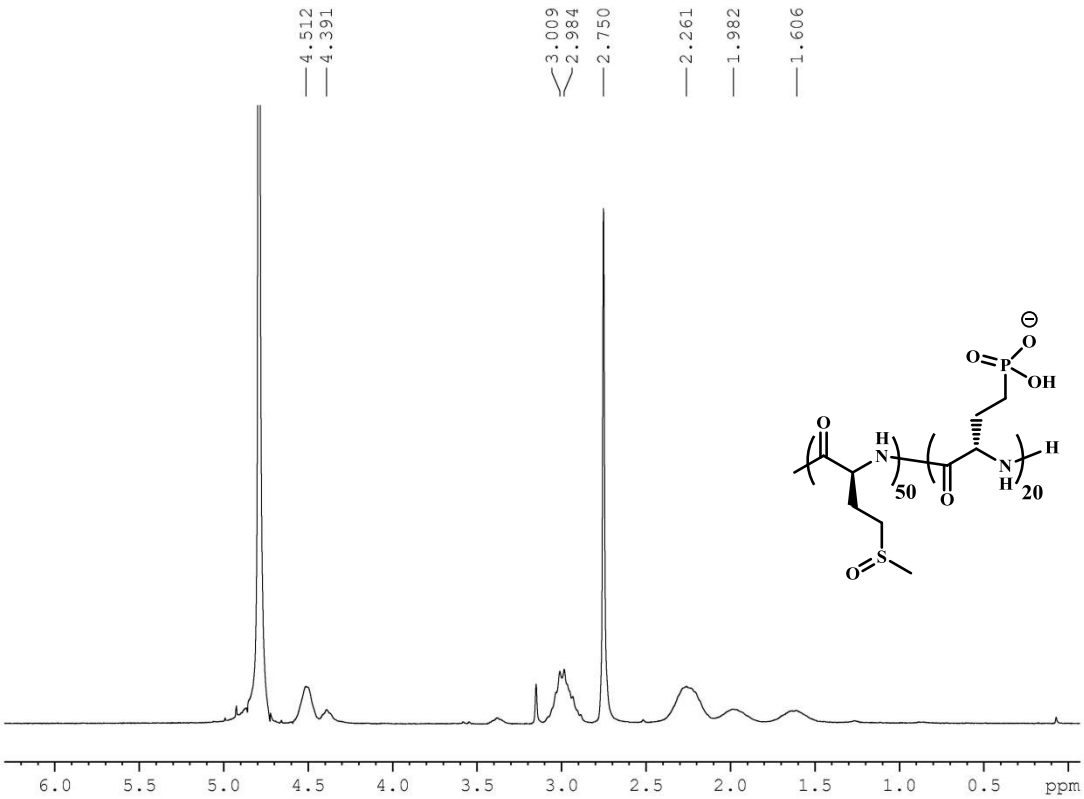
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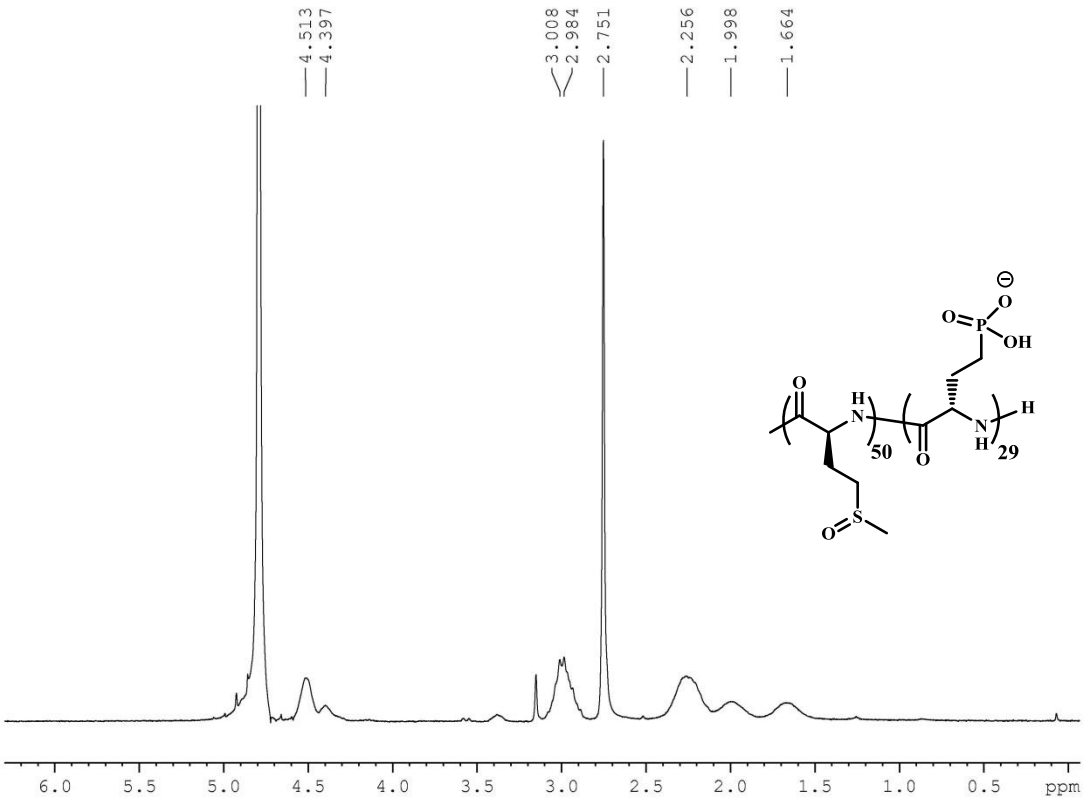
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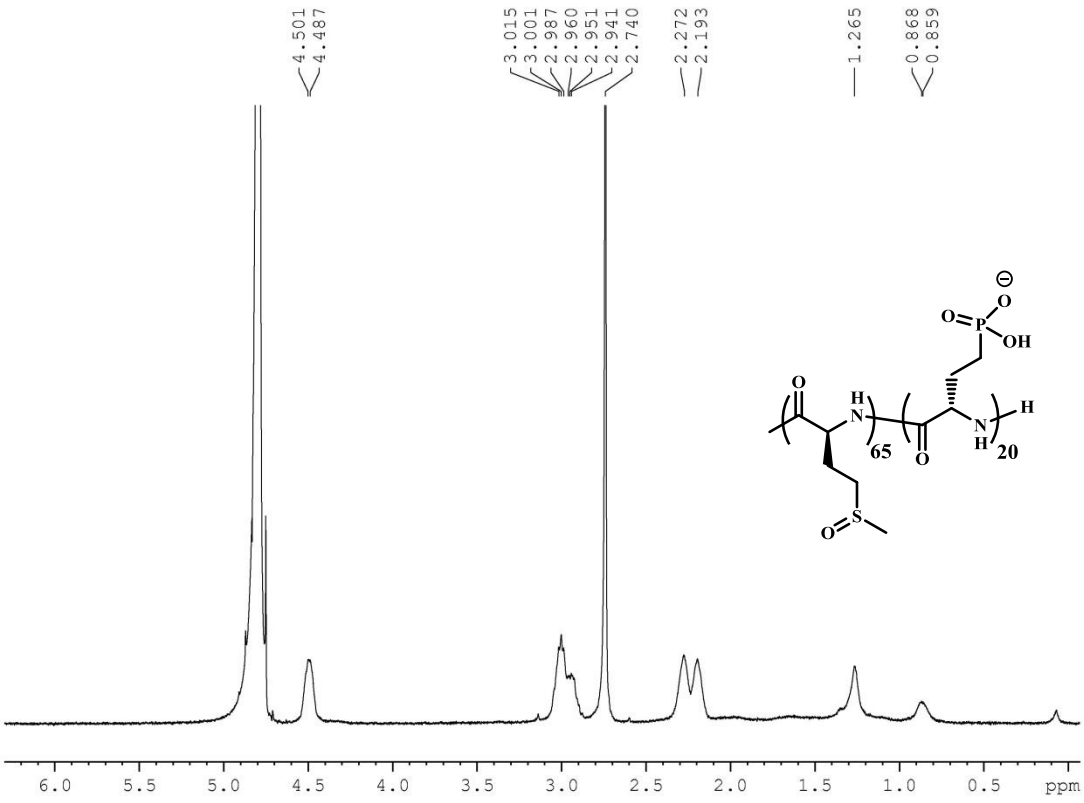
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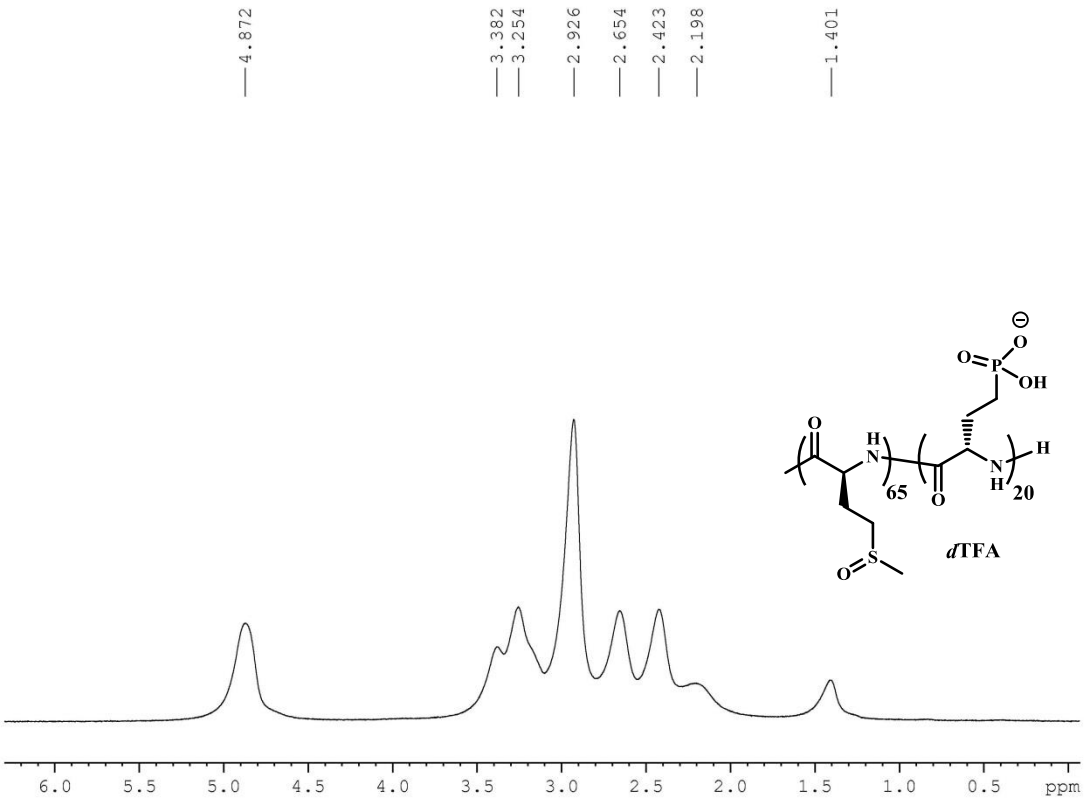
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CHAPTER FOUR: Phosphatidylcholine-Containing Polypeptides

4.1 Introduction

Phospholipids are ubiquitous in biology and are the majority components of the cell membrane. The phospholipid backbone is composed of glycerol, which links to two fatty acid tails and one hydrophilic head group. The resulting amphiphilic nature of the molecules allows them to self-assemble into a diverse array of nanostructures. Phospholipids owe their importance in biology due to their modular design; fatty acid tails and polar head groups may be varied depending on biological need.

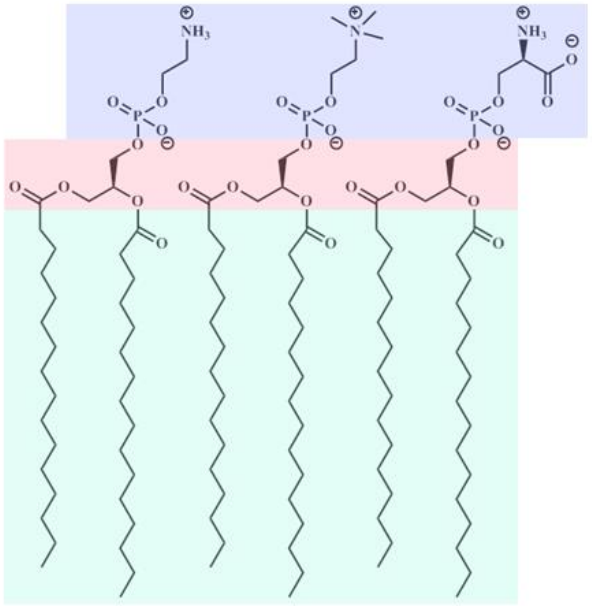


Figure 4.1. Phospholipids functionalized with different polar head groups. Headgroups from left to right: phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine.

Phosphatidylcholines are phospholipids that use choline as the hydrophilic headgroup; they are a major constituent of the outer leaflet of cell membranes.[102] Liposomes assembled from synthetic lipids have been thoroughly examined as drug delivery vehicles.[103-105] These liposomes

tend to not illicit an immune response due to their surface similarity to cells,[106] and often have low toxicity as they derived from natural building blocks. Unfortunately, poor liposome stability arising from the small size of individual building blocks continues to be a major problem. In addition small assembly sizes make phospholipid liposomes highly susceptible to renal clearance. To combat these issues, researchers turned to coating their liposomes with protective polymers.[107] The polymers coatings increase bulk and assembly stability, but they also introduce unnatural functionality, which may cause toxicity and biodegradability problems. In contrast to liposomes, vesicles assembled from polymers can overcome stability and renal filtration issues due to their larger size. However, polymer building blocks still suffer from toxicity and biodegradability issues.

A small number of researchers have started working with phosphatidylcholine-modified polymers in order to take advantage the polymer vesicle stability while mimicking the inocous nature of liposomes.[108-112] Poly[2-(methacryloyloxy)ethylphosphorylcholine], or PMPC, is one polymer that has been gaining traction. PMPC is easily produced and can be incorporated into diblock copolymers capable of self-assembly into polymersomes. Furthermore, these polymers can be tuned to respond to pH and possibly other triggers.[113] PMPC assemblies are minimally toxic,[113] but the polymers have a hydrocarbon backbone and are not biodegradable.

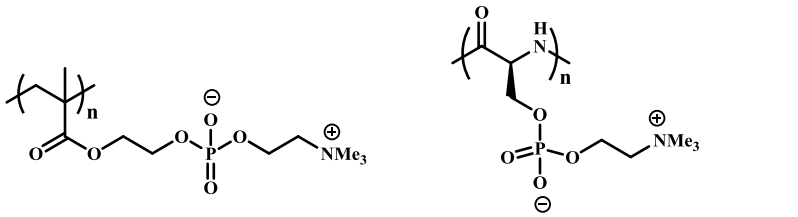


Figure 4.2. Structures of Poly[2-(methacryloyloxy)ethylphosphorylcholine] (PMPC) and poly(phosphatidylcholine serine).

Poly(phosphatidylcholine serine) sparked our interests due to its structural similarity to both phospholipids, and PMPC. Unlike PMPC, poly(phosphatidylcholine serine) is a polypeptide, and is expected to be biodegradable. We also expect poly(phosphatidylcholine serine) to have low toxicity due owing to its natural composition. The phosphatidylcholine serine amino acid is known to serve a number of vital roles in intracellular functions,[114] and is a building block for some phospholipids.[114-116] Development of a scalable and reliable synthetic method toward poly(phosphatidylcholine serine) and similar polypeptides would have immense value in drug delivery research and perhaps in the clinic.

4.2 Synthesis of 2-Bromoethylmethylphosphoserine and 2-Bromoethyl-2-Chlorophenylphosphoserine NCAs

Based on synthetic work toward phosphonate-containing NCAs discussed in Chapter 2, we knew that the selection of proper protecting groups would once again be of high importance. After some exploratory studies, we decided to use the 2-bromoethyl group as a choline synthon, leaving the need for an additional protecting group on the phosphate moiety. Similar trialkyl phosphates have been elaborated to phospholipid headgroup-like functional groups, albeit in a different context.[76] The protected NCA would be polymerized, then modified, and finally deprotected to yield the desired poly(phosphatidylcholine serine) (Figure 4.3).

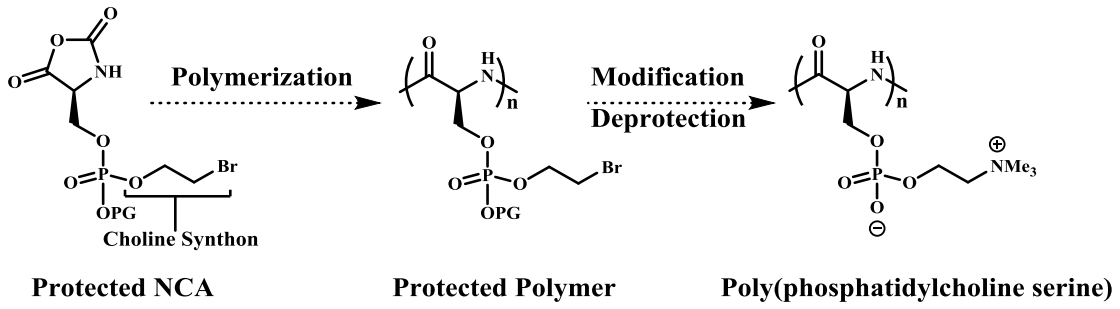


Figure 4.3. Synthetic strategy toward poly(phosphatidylcholine serine).

The beginning stages of this project took place concurrently to the dialkylphopshoserine synthetic studies discussed in the beginning of Chapter 2. Being unaware of the solubility issues discussed earlier, we chose to use the methyl protecting group for our first attempt.

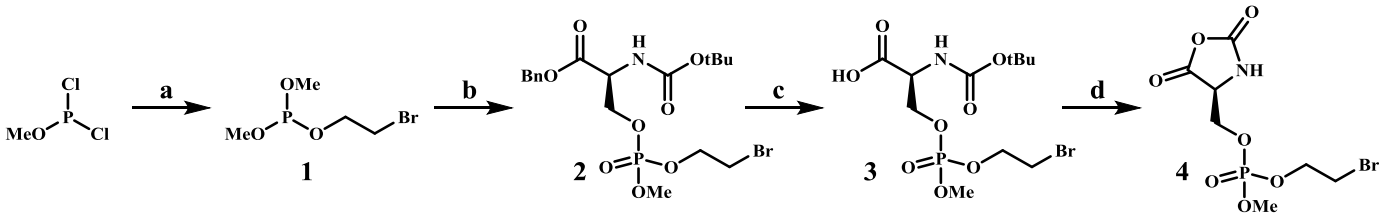


Figure 4.4. Synthesis of 2-bromoethylmethylphosphoserine NCA, **4**. (a) 2-bromoethanol, DIPEA, then MeOH (37% yield); (b) **1**, CBr_4, pyr. 0°C→rt (84% yield); (c) H_2, MeOH, Pd/C (72% yield); (d) various conditions.

We began our synthesis with preparation of the phosphite reagent **1**, which was elaborated to the modified protected serine derivative **2**. Both the α-amine and α-carboxylate needed to be protected for this reaction to work properly. The α-carboxylate of **2** was deprotected via hydrogenolysis to yield the NCA precursor **3**. The NCA **4** was made using Ghosez reagent, dichloromethyl methyl ether, or phosphorus tribromide. As with DMPS (Chapter 2) the 2-bromoethylmethylphosphoserine NCA and its polymers suffered from poor solubility.

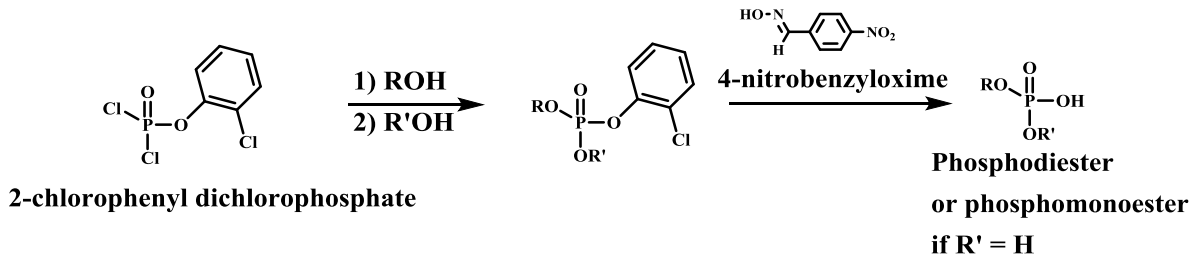


Figure 4.5. Synthetic strategy utilizing 2-chlorophenyl dichlorophosphate.

We then decided to explore the utility of bulkier, more hydrophobic phosphate protecting groups. 2-chlorophenyl has been used as a protecting group in syntheses of phosphomonoesters and phosphodiesters.[117-120] The precursors were easily synthesized from the commercially available 2-chlorophenyl dichlorophosphate, and were deprotected by 4-nitrobenzyloxime[121] or other reagents[117]. This route was attractive to us due to stability of the 2-chlorophenyl group and its facile deprotection (Figure 4.5).

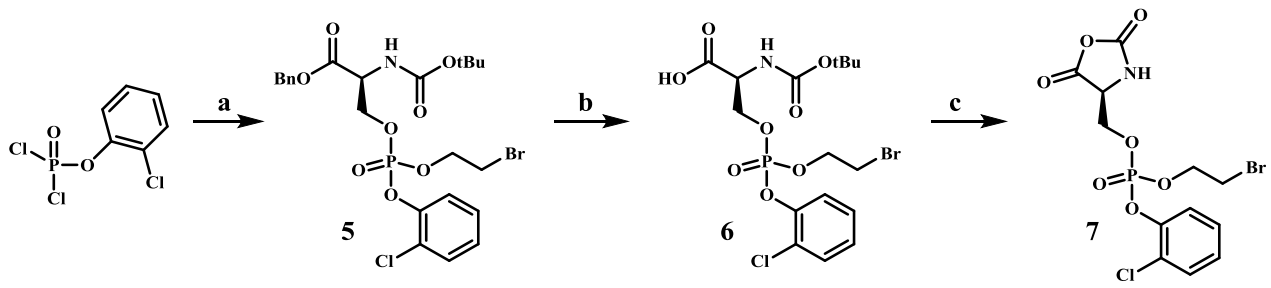


Figure 4.6. Synthesis of 2-bromoethyl-2-chlorophenylphosphoserine NCA, **7**. (a) Boc-Ser-OBn, pyr, then 2-bromoethanol (92% yield); (b) H_2, Pd/C, MeOH (60% yield); (c) Ghosez reagent, THF, 36 h (50% yield).

We started with the commercially available 2-chlorophenyl dichlorophosphate, which was elaborated to the protected serine derivative **5**. The α-benzyl protecting group was removed by hydrogenolysis, yielding **6**, which was then converted to the NCA **7** using Ghosez reagent (Figure 4.6). As with previous syntheses of phosphorylated serine NCAs, this route was plagued by inability to purify the monomer and efforts to polymerize the NCA were fruitless.

4.3 Synthetic Strategies for Rapid Testing of Phosphoserine Protecting Groups

Previous failures in synthesis of both dialkylphosphoserine NCAs and 2-bromoethyl-alkyl phosphoserine NCAs made it clear that we needed a more modular approach toward proper protecting group identification. Our efforts were shifted to the development of a route which could accommodate installation of various protecting groups into the phosphate moiety instead of focusing on one derivative at a time. We started by exploring phosphorus (V) oxychloride

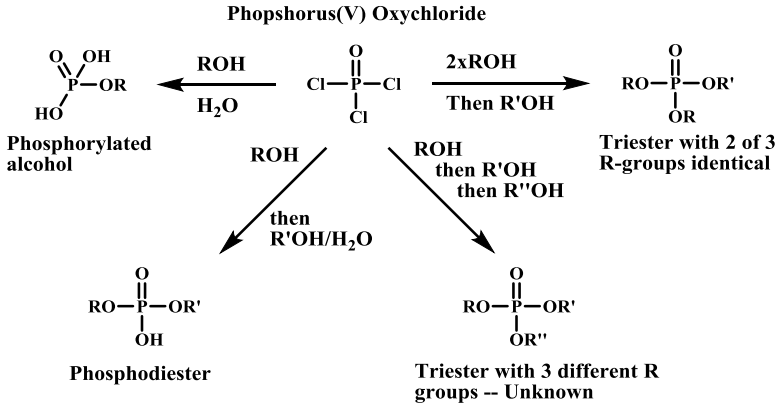


Figure 4.7. Substituted phosphates from phosphorus (V) oxychloride.

as a precursor to phosphate triesters. Phosphorus (V) oxychloride has been utilized as a phosphorylating reagent for quite some time,[122,123] and has been elaborated to phosphodiesters directly or phosphotriesters stepwise.[124] However, the direct synthesis of phosphotriesters with 3 different alkyl groups was unknown in literature when this work took place. Nevertheless, we decided to test this as the payoff would have been worthwhile.

The *tert*-butyl has been used in the synthesis of protected phosphoserine derivatives,[125] so we chose to incorporate it in our test reactions. Unsurprisingly, the desired phosphoserine triester

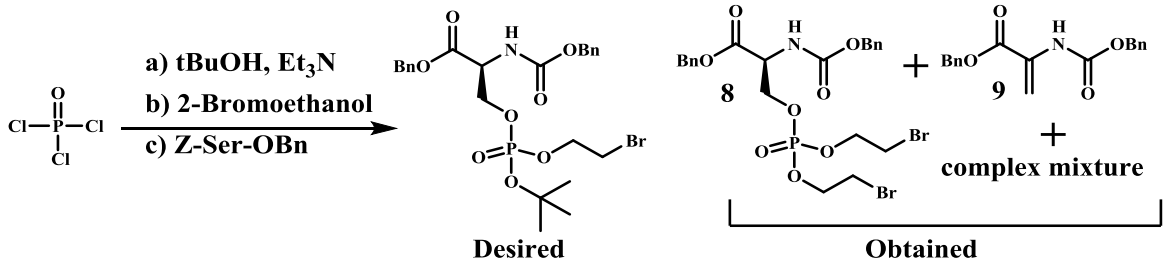


Figure 4.8. Attempted direct synthesis of phosphotriesters from phosphorus (V) oxychloride.

was not obtained. Instead the reactions yielded mixtures of the di-2-bromoethyl adduct **8**, dehydroalanine derivative **9**, and other serine derivatives, which were not indentified (Figure 4.8). 2-bromoethyl dichlorophosphite **10** was then prepared in order to circumvent side

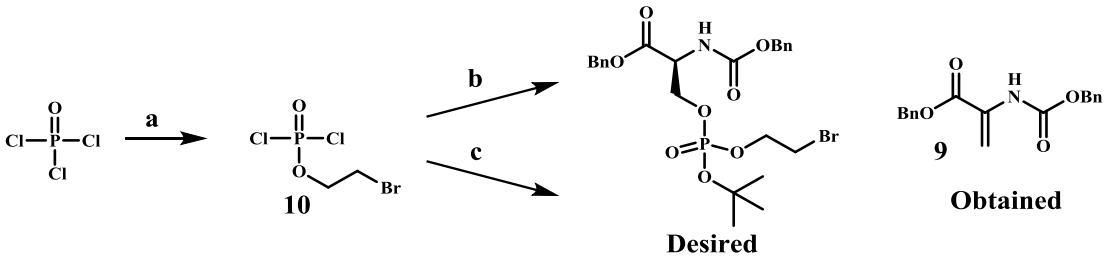


Figure 4.9. Attempted stepwise synthesis of phosphotriesters. (a) 2-bromoethanol, DCM, r. t. 18h (46% yield); (b) Z-Ser-OBn, Et_3N, then tBuOH; (c) tBuOH, Et_3N, then Z-Ser-OBn.

reactions that were observed in direct synthetic attempts. Only low yields of **9** were obtained via this approach, regardless of the order of addition. We theorized that *tert*-butanol was too bulky to yield the desired product, so we decided to use the less bulky 2-trimethylsilylethyl,[126] which has been used as a protecting group in oligonucleotide synthesis. Unfortunately, switching to a less bulky protecting group still gave the undesired derivate **9**; changing the order of addition had no effect (Figure 4.10).

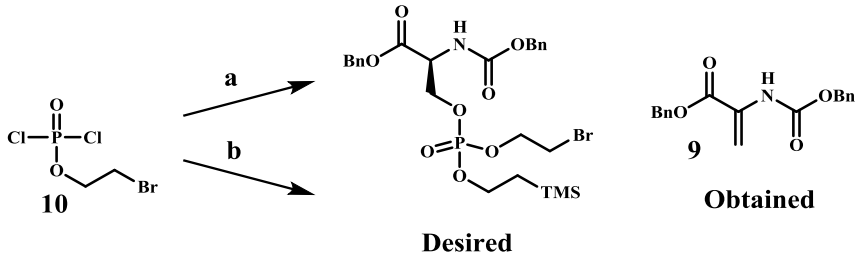


Figure 4.10. Attempted stepwise synthesis of phosphotriesters with reversed order of addition. (a) Z-Ser-OBn, Et_3N, then 2-trimethylsilylethanol; (b) 2-trimethylsilylethanol, Et_3N, then Z-Ser-OBn.

It became clear that routes utilizing alkyl dichlorophosphate reagents were too problematic for our purposes. We then looked to phosphite reagents, which are analogous in concept to phosphate reagents but tend to be amenable to triple substitution. One difference is the oxidation state of phosphorus, which is in a +3 state in phosphites, and must be oxidized to phosphorus (V) in order to complete the synthesis (Figure 4.11).

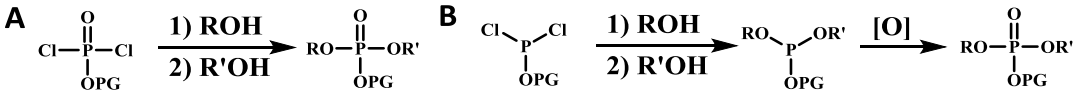
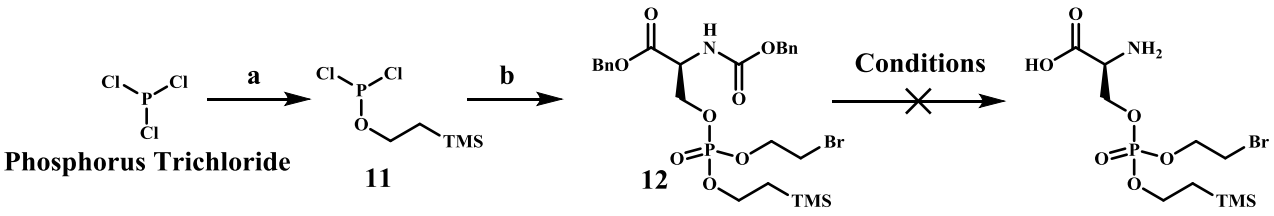


Figure 4.11. Differences between dichlorophosphate approach (a) and dichlorophosphite approach (b).

Using previously established methodologies, 2-trimethylsilylethyl was installed onto the commercially available phosphorus trichloride.[127,128] The resulting reagent **11** was treated with Z-Ser-OBzl in the presence of Hunig's base, followed by treatment with 2-bromoethanol. The phosphite intermediate was then oxidized with aq. 70% tert-butyl hydroperoxide, yielding the desired derivative **12**. The order of addition is important, as treatment of 2-bromoehanol followed by Z-Ser-OBzl results in a complex mixture. Unfortunately all consequent attempts to obtain the deprotected amino acid failed, and the derivative could not be elaborated to the NCA (Figure 4.12).



Conditions	Result
H_2, Pd/C, MeOH	Loss of phosphate protecting group
H_2, Pd/C, iPrOH	Loss of phosphate protecting group
H_2, Pd/C, EtOAc	No reaction
H_2, Pd/C, THF	No reaction
NiCl, $NaBH_4$, MeOH[129]	Loss of phosphate protecting group

Figure 4.12. Attempted synthesis of 2-bromoethyl-2-trimethylsilylphosphoserine. (a) 2-trimethylsilylethanol, -40→0°C, Et_2O (58% yield); (b) Z-Ser-OBn, DIPEA, then 2-bromoethanol, then tBuOOH (70% yield).

Figure 4.12 presents a 3 step preparation of NCA precursors with a variable protecting group. The route is not ideal due to early installation of the protecting group, incompatibility with hydrogenolysis, and somewhat low overall yields. Early 2-bromoethyl incorporation would give the 2-bromoethyldichlorophosphite as the starting reagent, shifting protecting group installation to a later stage. The somewhat low yields at the phosphotriester step likely stemmed from poor selectivity. The selectivity in related systems has been enhanced by use of phosphoramidites, which react at the amidite only with the addition of an appropriate base (Figure 4.13 A and B).[130,131]

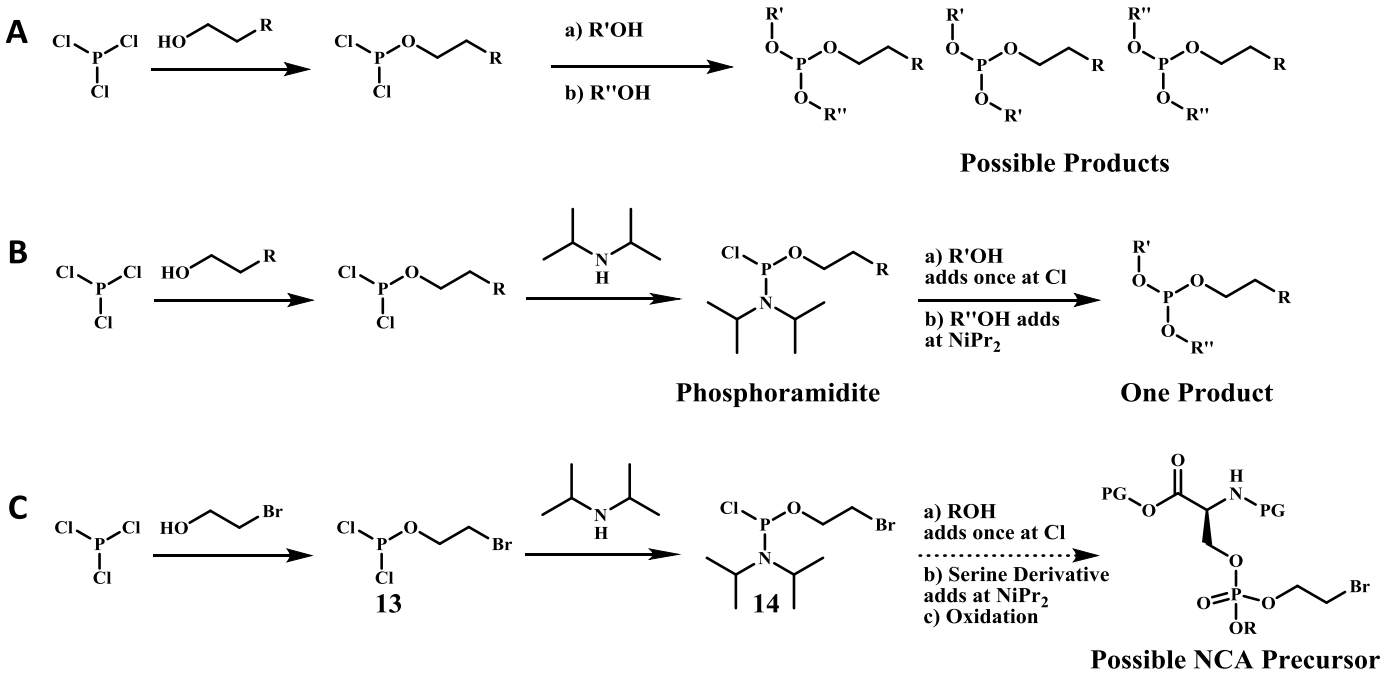


Figure 4.13. The dichlorophosphite method (A), the phosphoramidite method (B), synthesis of our reagent **14** (C).

The phosphoramidite reagent was synthesized by reacting phosphorus trichloride and 2-bromoethanol, which proceeded in 97% yield. One of the chlorides was then replaced with diisopropylamine, providing the chlorophosphoramidite **14** in 74 % yield. A protecting group can be added to the reagent followed by a serine derivative. This route would yield the NCA precursor in less steps and higher overall yield (Figure 4.13 C). Furthermore, this route would allow for faster screening of protecting groups due to their incorporation in the last step of reagent synthesis.

4.4 Synthesis and Polymerization of 2-Bromoethyl-Benzylphosphoserine NCA

With phosphoramidite **14** in hand, we then altered our route to allow for arger selectability of protecting groups. Our previous synthesis used a hydrogenolysis step in order to prepare the NCA precursor; this precluded us from using common protecting groups such as 2-cyanoethyl

and benzyl on the phosphate moiety as they aren't compatible with hydrogenolysis. In order to circumvent this problem, we decided to use a silyl ester to protect the carboxylate in situ,[132] which would allow us to remove an extra step, perform the phosphorylation in one reaction, eliminate the need for a separate α-carboxylate protection step.

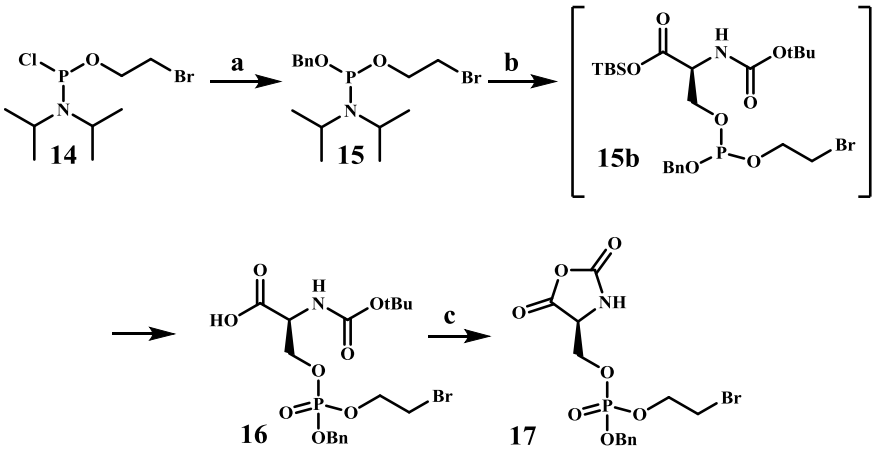


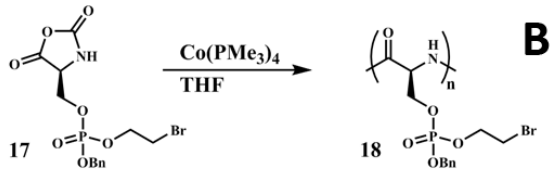
Figure 4.14. Synthesis of 2-bromoethylbenzylphosphoserine NCA, **17.** (a) BnOH, DIPEA, THF, 0°C (91% yield); (b) TBSCl, THF, Boc-Ser-OH, N-methylmorpholine, then **15**, tetrazole, then tBuOOH (95% yield). (c) Phosgene, N-methylmorpholine, THF (63% yield).

We chose to use benzyl as a phosphate protecting group due to its stability, hydrophobicity, and simple removal. The group was installed by reacting **14** with benzyl alcohol, yielding the dialkyl phosphoramidite **15** with high efficeincy. The next step was accomplished by protecting the α-carboxylate of Boc-Ser-OH with tertbutyldimethylsilyl chloride *in situ*, followed by reacting the side chain of Boc-Ser-OH with **15**. The intermediate phosphite **15b** was then oxidized using tert-butyl hydroperoxide and the α-carboxylate silyl group was removed during workup. The desired derivative **16** was obtained in pure form and with excellent yield (Figure 4.14). **16** was stable to aqueous acid/base workups, while the benzyl group was highly susceptible to acid in organic solution. Using dichloromethyl methyl ether for NCA synthesis resulted in the loss of the benzyl groups. Following optimization, the NCA **17** was successfully synthesized using Ghosez reagent or phosgene in the presence of N-methylmorpholine.

4.5 Synthetic Studies Toward Poly(Phosphatidylcholine Serine)

Polymerizations of NCA **17** using $Co(PMe_3)_4$ in THF proceeded rapidly at ambient temperature to give the corresponding polymer **18** with no reactions at the side chain phosphate groups. We were able to achieve full monomer conversion degrees of polymerization of up to 180. In order to gauge chain length control, **17** was polymerized to completion at different M:I ratios, and the active chains were then end-capped with isocyanate terminated PEG (PEG-NCO, M_n = 1000 Da).[87] The chains were then purified and analyzed by ^{1}H NMR to provide the number average molecular weights, which increased linearly with M:I stoichiometry (Figure 4.15).

A



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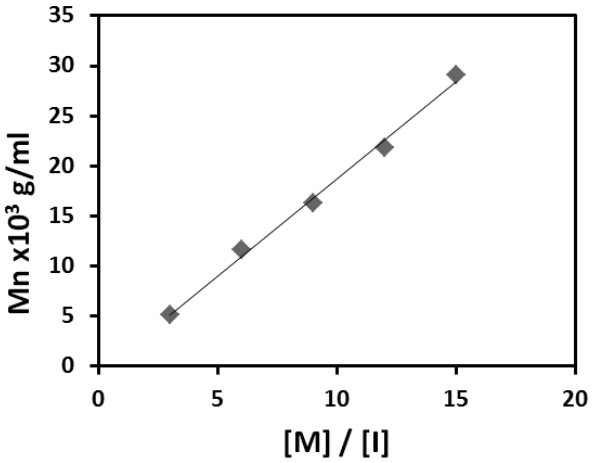


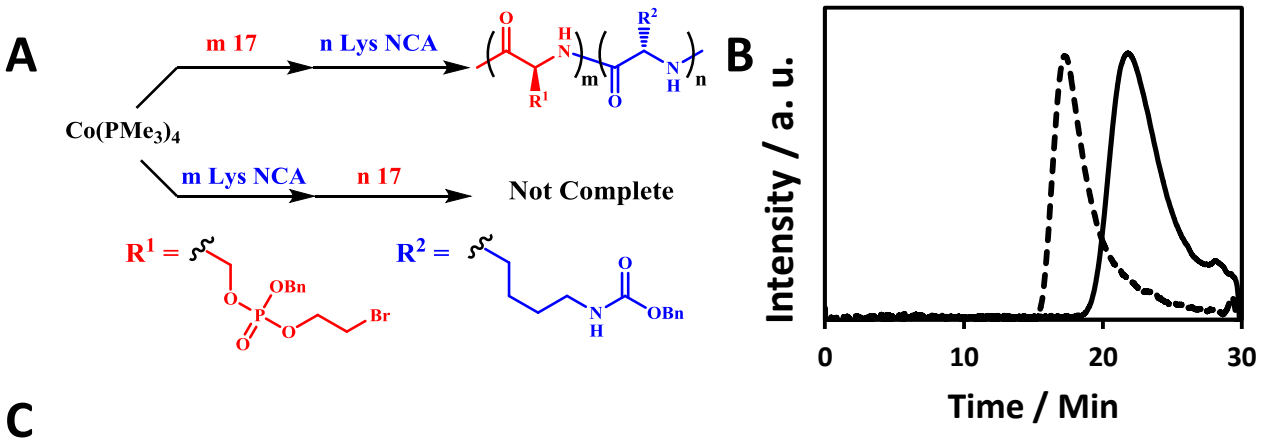
C

M:I[a]	Mn[b]	M_w/M_n[c]	DP[d]	yield (%)[e]
3:1	5 100	n/a	15	90
6:1	11 648	n/a	32	89
9:1	16 280	n/a	45	92
12:1	21 840	n/a	60	90
15:1	29 120	n/a	80	89

Figure 4.15. Summary of polymerizations of 2-bromoethylbenzylphosphoserine NCA, **17.** (A) Synthesis of poly-(2-bromoethyl-benzyl phosphoserine) **18**. (B) Plot of molecular weight (M_n) vs monomer to initiator ratio. (C) Synthesis of polymers **18** of different lengths. [a] Number of equivalents of monomer per $Co(PMe_3)_4$. [b] Molecular weight of first segments determined for PEG end-capped samples using ^{1}H NMR. [c] Polydispersity index of the polymers. [d] DP = degree on polymerization. [e] Isolated yield. n/a = not applicable.

The polypeptides were isolated in high yields with predictably controlled chain lengths of up to 80 residues. These high molecular weights were obtained after reaction times of less than 12 hours. Chain lengths of 15 to 80 are within a desirable range for many polypeptide material

applications.[39] Overall, these data show that NCA **17** undergoes controlled polymerization when initialed with $Co(PMe_3)_4$.



C

		First Segment			Diblock Copolymer			
First Monomer	*Second Monomer*	M_n[a]	M_w/M_n[b]	*DP*[c]	M_n[a]	*Mw/Mn*[b]	*DP*[c]	*yield (%)*[d]
9 NCA **17**	45 Z Lys NCA	11 600	1.47	45	84 300	1.34	304	90

Figure 4.16. Diblock copolypeptides containing 2-bromoethylbenzylphosphoserine. (A) Schematic representation of diblock synthesis; (B) GPC chromatogram (RI intensity in arbitrary units (au) versus elution time) of poly(2-Bromoethyl-Benzylphosphoserine) (solid), and poly(2-bromoethyl-benzylphosphoserine)-*block*-poly(Z-Lys). (C) Diblock copolymer data. [a] Molecular weight of first segments determined for PEG-NCO (1 kDa) end-capped samples using ^{1}H NMR. [b] Polydispersity index of the polymers. [c] DP = degree of polymerization. [d] Isolated yield.

The utility of this monomer is further exemplified in the successful synthesis of a diblock copolymer combined with Lys NCA as a model comonomer (Figure 4.16). We found that polymerizations only proceeded to completion when **17** was used in the first block and not vice versa. This is likely due to small amounts of impurities in the monomer. This is especially evident when initiation efficiency, or molecular weight inflation, of homopolymers **18** is analyzed. When polymerized, monomer **17**, displays a consistent molecular weight inflation of about 5 times greater relative to what would be expected at 100% efficiency (Figure 4.15B). It is

likely that impurities which decrease initiation efficiency also hamper growth of the second block when Lys NCA is used as the first segment.

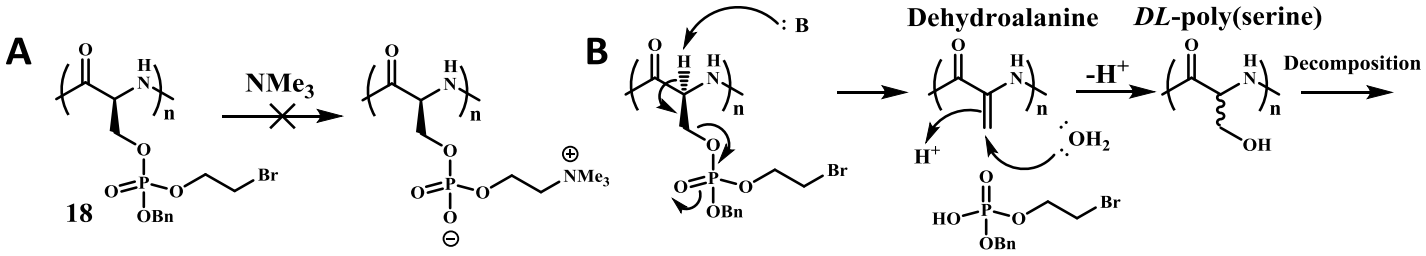


Figure 4.17. (A) Attempted synthesis of poly(phosphatidylcholine serine) **19**; (B) Proposed mechanism of decomposition

We then attempted the transformation of **18** to the desired poly(phosphatidylcholine serine) (Figure 4.17A). We hoped that due to the known short half-lives of phosphotriesters in aqueous solution,[133] we would be able to remove the labile benzyl and modify the 2-bromoethyl group to choline and in one step. Thus, we subjected **18** to a 20% aqueous solution of trimethylamine to find that the polymer went from an insoluble solid to a clear solution in about 10 minutes. Unfortunately upon dialysis, no product was isolated. Further experimentation showed that this effect only occurred in strongly basic solution, and did not require the presence of trimethylamine. These results suggest that peptide backbone cleavage was occurring. One way this could be happening is via β-elimination of the phosphate group yielding dehydroalanine,[134-136] followed by *in situ* formation of poly(*DL*-serine), which is known to decompose under basic or acidic conditions (Figure 4.17B).[137]

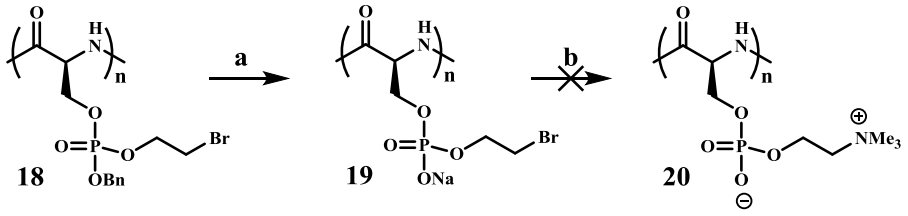


Figure 4.18. Synthesis attempts of poly(phosphatidylcholine serine) **20**. (a) 1:1 TFA/DCM (95% yield); (b) aq. 20% NMe_3 (decomposition).

We theorized that deprotecting the benzyl group would suppress the base-induced degradation of **18**, as the phosphodiester might be less susceptible to β-elimination. After screening a number of conditions, we found that the benzyl group can be removed using a 1:1 solution of TFA and DCM. Full conversion was achieved after stirring the polymer in TFA/DCM of 3 hours. Following purification by dialysis, the sparingly water soluble polymer **19** is obtained (Figure 4.18). Treatment of **19** with aqueous 20% trimethylamine followed by dialysis once again resulted in decomposition. It became clear that our synthetic route toward **20** had issues which would not be easily rectified with small changes to the method.

4.6 Synthetic Studies Toward Poly(Phosphatidylcholine Homoserine)

One possible approach to avoiding polymer degradation is by extension of serine's side chain to its one carbon homolog, homoserine. β-elimination on serine derivatives yields dehydroalanine, which contains a stabilized α,β-unsaturated olefin, while homoserine derivatives do not benefit from this effect (Figure 4.19).

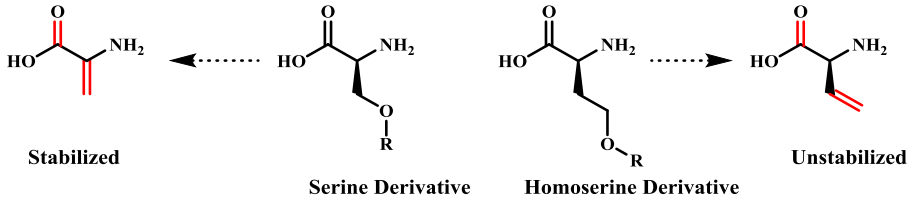


Figure 4.19. β-elimination of serine and homoserine derivatives showing the formation of stabilized dehydroalanine and unstabilized vinyl glycine.

The homoserine derivative of poly(phosphatidylcholine serine) was synthesized using the synthetic route from section 4.4. Boc-*L*-homoserine **21** was synthesized from the commerctially available *L*-homoserine. **21** was functionalized using phosphoramidite **15**, and subsequent oxidation provided **22** in excellent yield. **22** was converted to the NCA **23** in good yield using Ghosez reagent (Figure 4.20).

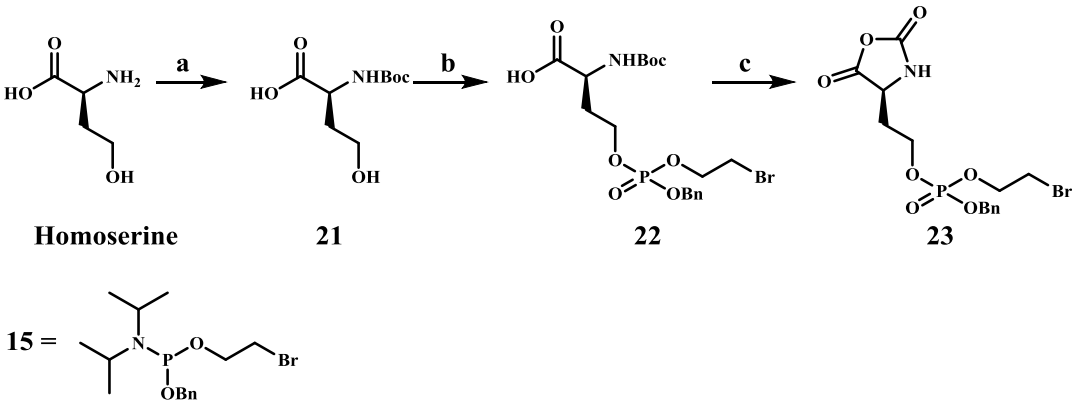
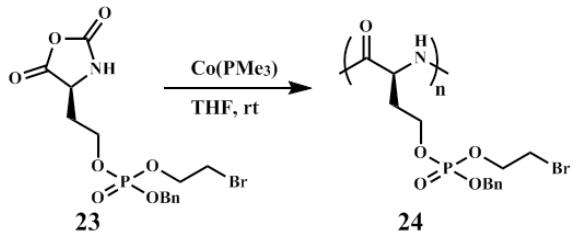


Figure 4.20. Synthesis of 2-bromoethylbenzylphosphohomoserine NCA, **23**. (a) Boc_2O, NaOH (91% yield); (b) TBSCl, THF, Boc-*L*-homoserine, N-methylmorpholine, then **15**, PPTS, then tBuOOH (95% yield); (c) Ghosez reagent, THF, 24h.

The NCA **23** was obtained as a clear colorless oil following silica chromatography. Polymerizations of the NCA using $Co(PMe_3)_4$ in THF proceeded readily at ambient temperature to give the corresponding homopolypeptides **24** with complete monomer conversions and no reactions at the side-chain phosphate groups (Figure 4.21A). Residual Co salts were removed by washing the polypeptides with water. In order to gauge if chain lengths could be controlled, **24** was polymerized to completion at different M:I ratios, and active chains were then end capped with isocyanate terminated PEG (PEG-NCO, M_n = 1000 Da).[87] The chains were purified and analyzed by ^{1}H NMR to provide the number average molecular weights, which increased linearly with M:I stoichiometry (Figure 4.21B). Chain length distributions samples of **24** were obtained by GPC/RI analysis and polydispersity indices were found to be between 1.14 and 1.24, indicating that well-defined polypeptides were formed (Figure 4.21 C and D).

A



B

M:I[a]	M_n[b]	M_w/M_n[c]	DP[d]	yield (%)[e]
10:1	12 800	1.19	34	89
20:1	28 300	1.24	75	91
30:1	35 900	1.21	87	90
40:1	49 100	1.14	130	95

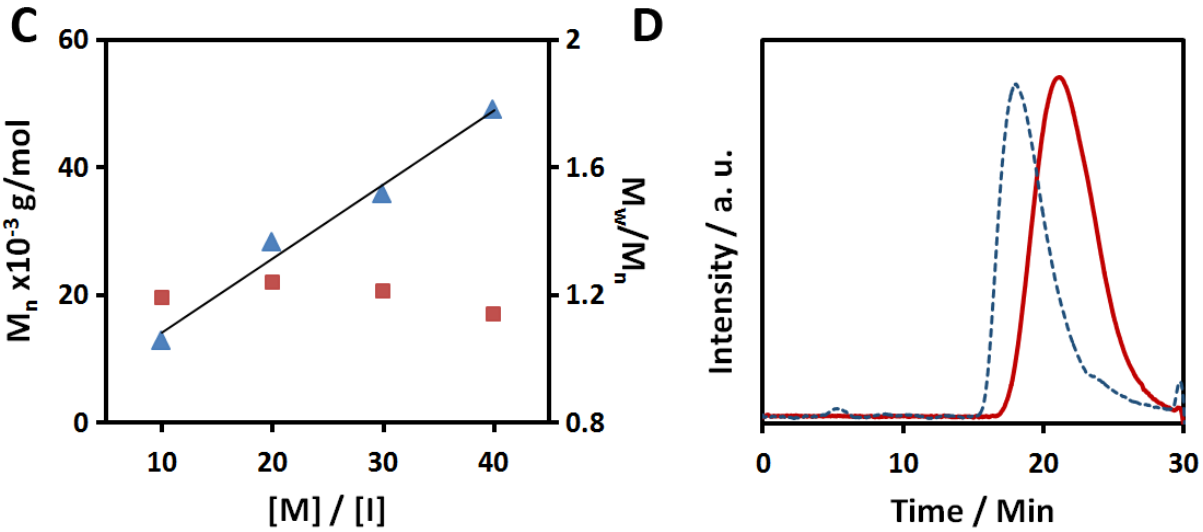


Figure 4.21. Summary of 2-bromoethylbenzylphosphohomoserine NCA polymerization results. (A) Scheme for the synthesis of poly(2-bromoethylbenzylphosphohomoserine), **24**. (B) Table summarizing polymer chain length data [a] Number of equivalents of monomer per $Co(PMe_3)_4$. [b] Molecular weight determined for PEG end-capped samples using ^1H NMR. [c] Polydispersity index determined by GPC/RI analysis. [d] DP = degree of polymerization. [e] Total isolated yield of purified polypeptide. (C) Molecular weight (M_n, ▲), and polydispersity index (M_w/M_n, ■) of **24** as a function of monomer to initiator ([M]/[I]) ratio using $Co(PMe_3)_4$ in THF at ambient temperature. (D) GPC Chromatogram (RI intensity in arbitrary units (au) versus elution time) of **24**, [M]/[I]=20:1, solid red; [M]/[I]=40:1, dotted blue.

Modification of **24** to poly(phosphatidylcholine homoserine) proceeded using 20% aq. trimethylamine in nearly quantitative yield. The polymer could also be obtained by first deprotecting **24** to yield **24b** (97 % deprotection), followed by modification of the 2-bromoethyl group to give **25** (Figure 4.22).

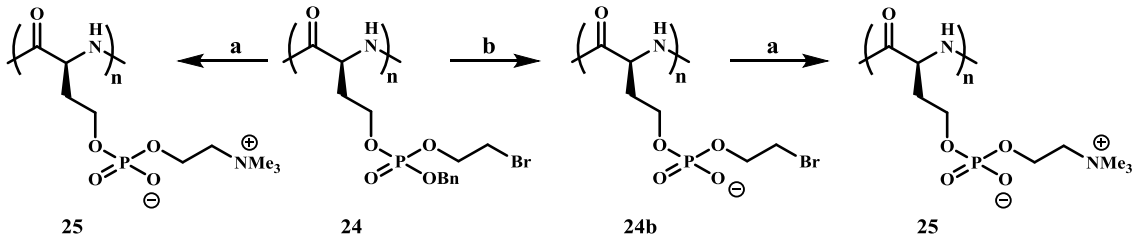


Figure 4.22. Synthesis of poly(phosphatidylcholine homoserine), **25**. (a) aq. 20% NMe_3, 4h (>90% yield); (b) 1:1 DCM:TFA, 4h (>95% yield).

4.7 Properties of Poly(Phosphatidylcholine Homoserine)

Poly(phosphatidylcholine homoserine) **25** is a zwitterionic polypeptide. Highly ionic polypeptides have been shown to primarily adopt random coil conformations,[60] which is what we expected to see with **25**. Our predictions were corroborated as **25** adopted disordered conformations over a range of pH as measured by circular dichroism (Figure 4.23).

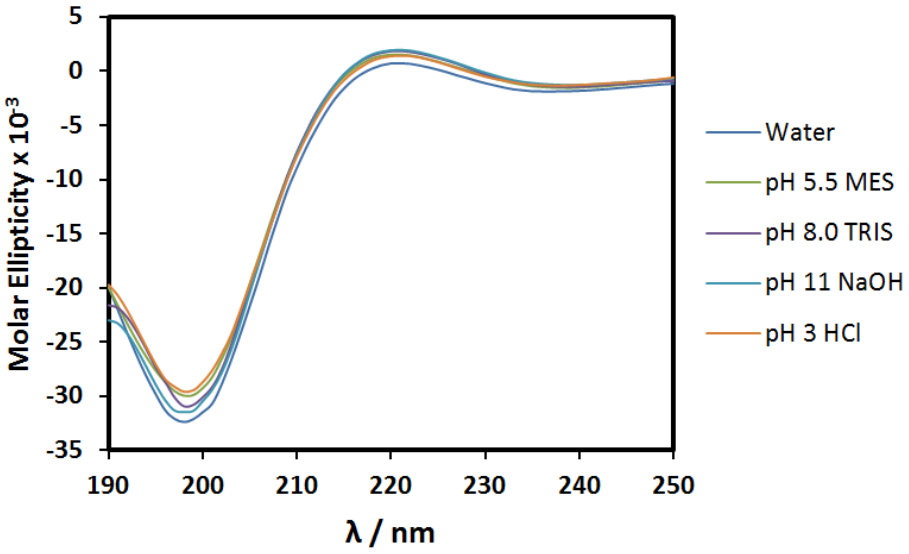


Figure 4.23. Circular dichroism spectra of poly(phosphatidylcholine homoserine), **25** (DP = 34, 0.1 mg/ml) at 20°C.

Phosphatidylcholine-containing polymers based on acrylic monomers display good biocompatibility.[138,139] Furthermore, similar polymers have been used as competent vectors for DNA delivery into mammalian cells.[112] Much like prior work, **25** is non-toxic in PC3 cells up to 5mg/ml (Figure 4.24).

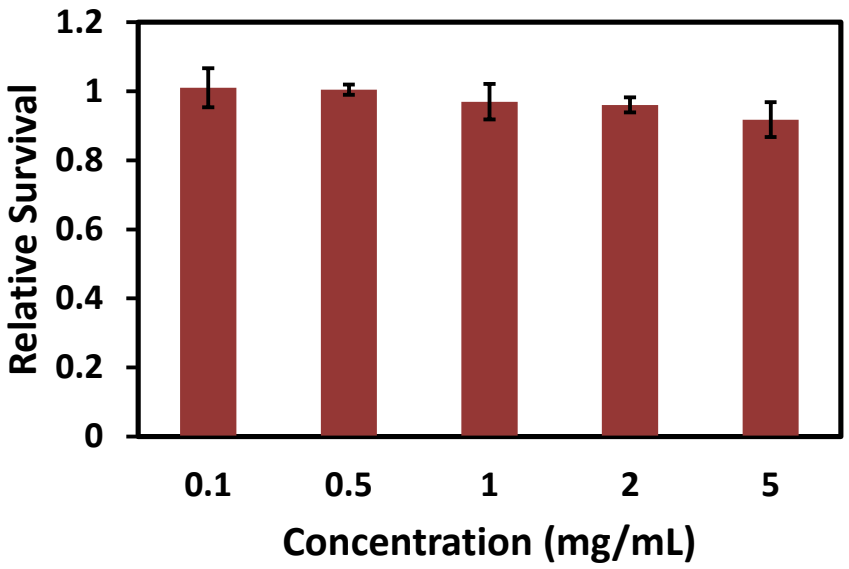


Figure 4.24. MTS cytotoxicity assay of poly(phosphatidylcholine homoserine), **25** at different concentrations using PC3 prostate cancer cells, 5 hour incubation, cell density = 60,000 cells/cm^2. Relative survival is the ratio of viability of cells exposed to polymer to viability of cells without polymer.

These new polymers are unprecedented as phosphatidylcholine-bearing polypeptides have not been synthesized in the past using any method. Furthermore, these biocompatible polypeptides may prove to be superior materials for biomedical research due to the inherent biodegradability of the polypeptide backbone.

4.8 Conclusion

This chapter describes both positive and negative results toward the synthesis of phosphatidylcholine-containing polypeptides. Successful synthesis of poly(phosphatidylcholine homoserine) is the first known preparation of a polypeptide bearing phosphatidylcholine groups. Similar non-biodegradable polymers have been produced in the past and show very promising results for drug delivery. Based on our cytotoxicity results and prior knowledge of polypeptide materials, we believe that poly(phosphatidylcholine homoserine) will not only bear the positive

properties of the aforementioned polymers, but will also be superior due to its intrinsic biodegradability.

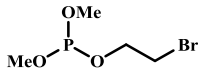
4.9 Experimental

4.9.1 Materials and Methods

Unless otherwise stated, reactions were carried out in oven-dried glassware under an atmosphere of nitrogen using anhydrous solvents. THF, hexanes, DCM, and toluene were purified by passage through activated alumina-packed columns under nitrogen. Triethylamine, N-methylmorpholine, and Hünig's base were distilled from CaH_2 and was stored over 4Å molecular sieves in an airtight container. All reagents were ordered from Aldrich, Spectrum, Alfa Aesar, Bachem, or CombiBlocks, and were used as received unless otherwise stated. ^{1}H NMR spectra were recorded at 500MHz on Bruker instruments; ^{13}C NMR spectra were recorded on 125MHz Bruker instruments with the solvent peak as internal reference. ^{31}P NMR spectra were recorded at 500MHz or 300MHz on a Bruker instrument using and internal 85% aqueous H_3PO_4 reference sealed in a glass capillary. Thin-layer chromatography (TLC) was conducted with EMD gel 60 F254 precoated plates (0.25 mm) and visualized using a combination of UV, permanganate, ninhydrin, and ceric ammonium molybdate staining. Selecto silica gel 60 (particle size 0.032–0.063 mm) was used for flash column chromatography. The silica was dried under vacuum at 200 ^{o}C for 2 days before it was used in the glove box. All Fourier Transform Infrared (FTIR) samples were prepared as thin films on NaCl plates and spectra were recorded on a Perkin Elmer RX1 FTIR spectrometer and are reported in terms of frequency of absorption (cm^{-1}). Gel permeation chromatography (GPC) was conducted using a Shimadzu high performance liquid chromatography (HPLC) system with a refractive index (RI) detector RID-10A, one Polymer Laboratories PLgel guard column, and two Polymer Laboratories PLgel 5 µm mixed D columns. DMF with LiBr (0.1 M) at 40^{o}C was used as eluent (flow rate: 0.60 ml/min). GPC/RI samples were prepared at concentrations of 10 mg/mL. Circular dichroism spectra were recorded

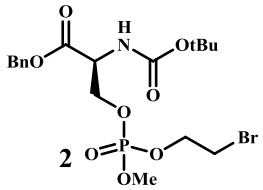
on an OLIS RSM CD spectrophotometer running in conventional scanning mode. Optical rotations were measured on a Rudolph Research Analytical Autopol III Automatic Polarimeter. NCA purifications and polymerization reactions were conducted in a nitrogen-filled glove box. Purified NCAs and cobalt initiator were stored in a -28 ^{o}C freezer inside of a glove box.

4.9.2 Experimental Procedures



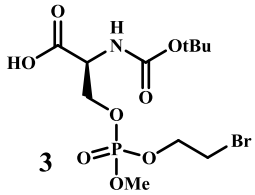
1

2-Bromoethyl dimethyl phosphite (1). The following procedure has been modified from previously published work.[76] 450 ml of dry THF were added via cannula to a 1 liter round bottom flask equipped with a stir bar. Methyl dichlorophosphite (9.70 g, 73.0 mmol) was then added to the flask via a syringe and the mixture was stirred and cooled to -78 ^{o}C under nitrogen. Diisopropyl ethyl amine (38.1 ml, 219 mmol) was then added to the solution via a syringe. The solution was then vigorously stirred and treated with 2-bromoethanol (5.20 ml, 73 mmol) dropwise. The stirring was continued for 1 hour, followed by the addition of dry methanol (2.95 ml, 73.0 mmol). The reaction was stirred for 30 more minutes at -78 ^{o}C and was then allowed to warm to room temperature. The solution was then diluted with 150 ml of diethyl ether and the solids were removed by filtration through celite. The solvent was removed using the rotovap, yielding a cloudy yellow liquid. The crude was then purified using vacuum distillation with the desired distillate coming through at 45 ^{o}C, yielding the product as a clear, colorless liquid (6.00 g, 27.7 mmol, 37%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.10 (2H, q, J=6.5 Hz), 3.56 (3H, s), 3.54 (3H, s), 3.49 (2H, t, J=6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 62.0 (d, J_{CP}=11.5 Hz), 49.3 (d, J_{CP}=10.5 Hz), 43.6 (impurity), 31.2 (d, J_{CP}=4.7 Hz).



2

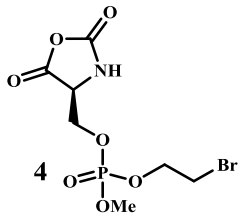
Benzyl *O*-((2-bromoethoxy)(methoxy)phosphoryl)-*N*-(*tert*-butoxycarbonyl)-*L*-serinate (2). The following procedure was based on previously published work.[77] Boc-Ser-OBn (1.51 g, 5.12 mmol) and carbon tetrabromide (3.74 g, 11.2 mmol) were dried under vacuum. The solids were then dissolved in 8ml of dry pyridine and the solution was cooled to 0 ^{o}C. **1** (1.67 ml, 7.68 mmol) was then added dropwise via a syringe as the solution was vigorously stirred. The reaction was then stirred for 30 more minutes at the same temperature followed by 2.5 hours at room temperature. The reaction was then diluted with 50 ml of diethyl ether and was washed with 1.0 M HCl (2x20 ml), followed by saturated $NaHCO_3$ (20 ml), followed by brine (20 ml). The organic layer was then dried over $MgSO_4$ and was concentrated to yield a red crude oil. The crude was chromatographed using 1:1 EtOAc:Hex as eluent yielding the product as a clear, pale yellow oil (2.13 g, 4.29 mmol, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (5H, br s), 5.52 (1H, br s), 5.21 (2H, q, *J*=9.8 Hz), 4.57 (1H, m), 4.48 (1H, m), 4.34 (1H, m), 4.23 (2H, q, *J*=6.2 Hz), 4.12 (EtOAc), 3.70-3.74 (3H, m), 3.47 (2H, t, *J*=6.05 Hz), 2.04 (EtOAc), 1.45 (9H, s), 1.26 (EtOAc).



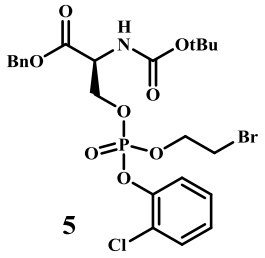
3

***O*-((2-bromoethoxy)(methoxy)phosphoryl)-*N*-(*tert*-butoxycarbonyl)-*L*-serine (3). 2** (3.40 g, 6.87 mmol) were dissolved in 20 ml of methanol. 340 mg of 10% palladium on carbon was then added to the flask and the resulting suspension was sparged with hydrogen 4 times. The reaction

was then stirred under a balloon of hydrogen for 5 hours, at which point the reaction was complete by TLC. The solution was then filtered through celite and the volatiles were removed under reduced pressure. The resulting oil was dissolved in 0.5 M K_2CO_3 and was washed with DCM (40 ml). The water layer was then acidified to a pH of ~2 with 1.0 M HCl and was once again extracted with DCM (4x25 ml). The washes from the second extraction were combined and were dried over $MgSO_4$. The solvent was removed under reduced pressure yielding the product as a clear, pale yellow oil. (2.00 g, 4.92 mmol, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.65 (1H, d, *J*=6.9Hz), 5.30 (DCM), 4.51 (2H, m), 4.34 (4H, m), 3.82 (3H, d, *J*=11.4 Hz), 3.53 (2H, t, *J*=6.1 Hz), 3.49 (MeOH), 1.45 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 155.5, 80.5, 60.1, 67.3 (d, J_{CP}=5.2 Hz), 55.1 (m), 53.7 (d, J_{CP}=7.3 Hz), 50.8, 44.7, 29.3 (t, J_{CP}=7.1 Hz), 22.5.

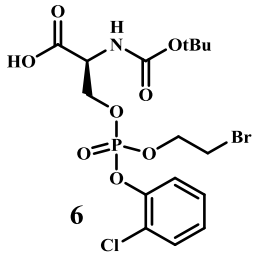


2-Bromoethyl methyl phosphoserine NCA (4). 3 (474mg, 1.17 mmol) was dissolved in 10 ml of dry DCM. Dichloromethyl methyl ether (210 µl, 2.33 mmol) was then added via a syringe and the reaction was refluxed under nitrogen for 36 hours. The resulting brown solution was filtered through a Schlenk frit and the solvent was removed under vacuum. The resulting brown oil was chromatographed in the glove box using 50/50 THF/Hexane as eluent yielding the NCA as a clear, pale yellow oil (50 mg, 0.15 mmol, 12% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (1H, d, *J*=15.1 Hz), 4.55 (1H, br s), 4.50 (1H, m), 4.41 (1H, m), 4.34 (2H, p, *J*=6.7 Hz), 3.83 (3H, d, *J*=11.4 Hz), 3.55 (2H, *J*=5.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.9 (d, J_{CP}=7.0 Hz), 151.7, 67.9 (Impurity), 67.9 (Impurity), 67.6 (dd, J_{CP}= 16.0, 5.5 Hz), 65.3 (t, J_{CP}=4.8 Hz), 58.3 (dd, J_{CP}= 5.9, 1.3 Hz), 55.3 (dd, J_{CP}=13.8, 6.3 Hz), 29.4 (t, J_{CP}=7.6 Hz), 25.6.



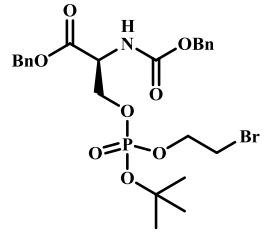
5

Benzyl *O* - ((2-bromoethoxy) (2-chlorophenoxy) phosphoryl) - *N* - (*tert*-butoxycarbonyl) -*L*-serinate (5). Pyridine (1.33 ml, 16.5 mmol) and 2-chlorophenyldichlorophosphate (475 µl, 2.89 mmol) were dissolved in 20 ml of dry DCM and the solution was cooled to 0 ^{o}C. In a separate vial, Boc-Ser-OBn (906 mg, 2.75 mmol) was dissolved in 20 ml of dry DCM and the solution was then slowly added to the reaction mixture. The reaction was stirred for 1 hour at 0 ^{o}C and was then removed from ice. 2-bromoethanol (252 µl, 2.75 mmol) was then added and the reaction was stirred at room temperature for 12 hours. 1 ml of water was then added to the reaction to quench any unreactred phosphochlorides and the solution was transferred to a separatory funnel. The organic layer was washed with 1.0 M HCl (2x20 ml) followed by brine (20 ml). The organic layer was then dried over $MgSO_4$ and was concentrated on the rotovap. The resulting cloudy oil was chromatographed in 50/50 EtOAc/Hex yielding **5** as a clear, colorless oil (1.50 g, 2.53 mmol, 92%, dr = 58:42). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12-7.43 (9H, m), 5.50 (0.43H, d, *J*=7.8 Hz), 5.40 (0.45H, d, *J*=8.0 Hz), 5.18 (2H, m), 4.61 (1H, d, *J*=5.5 Hz), 4.49 (2H, m), 4.36 (2H, m), 4.11 (EtOAc), 3.56 (0.84H, t, *J*=6.1 Hz), 3.48 (1.16H, t, *J*=6.1Hz), 2.04 (EtOAc), 1.43 (9H, s), 1.26 (EtOAc); ^{13}C NMR (125 MHz, $CDCl_3$) δ171.1, 168.9, 155.1, 146.2, 134.9, 130.7, 128.6, 128.6, 128.0, 126.3, 125.5, 121.5 (d, J_{CP}=2.4 Hz), 80.5, 68.8, 67.8, 67.8, 60.4, 53.9, 28.8-29.0 (m), 28.3, 21.0, 14.2.



6

***O*-((2-bromoethoxy)(2-chlorophenoxy)phosphoryl)-*N*-(*tert*-butoxycarbonyl)-*L*-serine (6).** **5** (1.50 g, 2.50 mmol) was dissolved in 15 ml of methanol and 150 mg of 10% Pd/C were added to the solution and the resulting suspension was sparged with hydrogen 4 times. The reaction was then stirred under a balloon of hydrogen overnight, at which point the reaction was complete by TLC. The solution was then filtered through celite and the volatiles were removed under reduced pressure. The resulting oil was dispersed in water and extracted with DCM (20 ml, 3 times). The DCM washes were combined and dried over $MgSO_4$, and the volatiles were removed under reduced pressure, yielding **6** as a pale yellow oil (800 mg, 1.59 mmol, 60%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (2H, m), 7.24-7.27 (1H, m), 7.15 (1H, m), 5.58 (1H, dd, *J*=13.8 Hz, 7.7 Hz), 4.66 (1H, m), 4.43-4.63 (4H, m), 3.53 (2H, q, *J*=5.8 Hz), 1.44 and 1.46 (9H, 2 diastereotopic signals); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 146.0, 130.8, 128.1, 126.5, 121.5, 80.8, 68.1, 53.6, 28.8, 28.3.



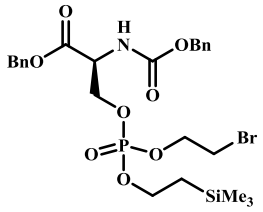
Attempted Synthesis

Benzyl *N*-((benzyloxy)carbonyl)-*O*-((2-bromoethoxy)(*tert*-butoxy)phosphoryl)-*L*-serinate

Phosphorus (V) oxychloride (2.00 ml, 21.3 mmol) was dissolved in 50 ml of dry hexane as the solution was cooled to -25 ^{o}C. A solution of t-butanol (2.00 ml, 21.3 mmol) and triethylamine

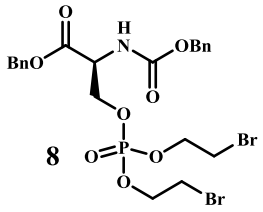
(3.00ml, 21.3 mmol) in 50 ml of hexane were then added via an addition funnel over 1 hour. The reaction was then removed from the cooling bath and was stirred for 2 hours. The mixture was cooled back down to -25 ^{o}C and was diluted with 60 ml of THF. A mixture of 2-bromoethanol (1.95 ml, 21.3 mmol) and triethylamine (3.00 ml, 21.3 mmol) in 50 ml of THF was then added dropwise over 1 hour. The reaction was then removed from the cooling bath and stirred for 2 hours. Z-Ser-OBzl (7.00 g, 21.3 mmol) and triethylamine (3.00 ml, 21.3 mmol) were then added as a mixture in 60 ml of THF). The reaction was stirred for an additional 2 hour. At this point, the starting material could no longer be detected by TLC. The excess reagents were quenched by adding 5 ml of water and the solids were filtered off. The excess water was removed with $MgSO_4$ and the solvent was removed under reduced pressure, yielding a clear orange oil. The oi was dissolved in 150 ml of EtOAc and was washed with water (40 ml), 10% $NaHCO_3$ (40 ml), followed by brine (40 ml). The organic layer was then dried over $MgSO_4$ and the solvents were removed under reduced pressure. The crude was then chromatographed using 5% diethyl ether in DCM as the eluent. The desired product was not obtained. Instead compounds **8** and **9** were isolated.

A similar procedure where the addition of Z-Ser-OBzl and tBu-OH were reversed yielded similar results. Using tBu-OK instead of tBuOH/Et_3N was not also not successful. The spectral data for **8** and **9** can be found below.



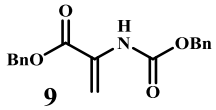
Attempted Synthesis

Benzyl *N*-((benzyloxy)carbonyl)-*O*-((2-bromoethoxy)(2-(trimethylsilyl)ethoxy)phosphoryl)-*L*-serinate. 10 (650 mg, 2.67mmol) was dissolved in 20 ml of dry DCM and cooled to 0 ^{o}C. Triethylamine (415 µl, 2.94 mmol) was added in one portion followed by 2-trimethylsilylethanol (385 µl, 2.67mmol). The mixture was stirred overnight and the ice bath was allowed to expire. The next day triethylamine (415 µl, 2.94 mmol) and Z-Ser-OBzl (880 mg, 2.67 mmol, in 10 ml dry DCM) were added. The solution was then brought to reflux for 3 hours. The excess reagents were quenched with 1ml of water, TLC showed a mixture of starting material and **9**. Reversal of addition of Z-Ser-OBzl and 2-trimethylsilylethanol yielded similar results.



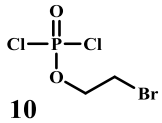
8

Benzyl *N*-((benzyloxy)carbonyl)-*O*-(bis(2-bromoethoxy)phosphoryl)-*L*-serinate (8). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (10H, br s), 5.81 (1H, d, *J*=8.1 Hz), 5.30 (DCM), 5.22 (2H, s), 5.13 (2H, d, *J*=3.1 Hz), 4.65 (1H, m), 4.51 (1H, m), 4.42 (1H, m), 4.23 (4H, m), 3.45 (4H, t, *J*=6.0 Hz).



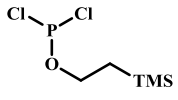
9

Benzyl 2-(((benzyloxy)carbonyl)amino)acrylate (9). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (10H, br s), 6.30 (1H, br s), 5.87 (1H, d, *J*=1.4 Hz), 5.27 (2H, s), 5.18 (2H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.5, 153.1, 135.8, 135.0, 128.6, 128.5, 128.5, 128.5, 128.3, 128.2, 106.3, 67.7, 67.0.



10

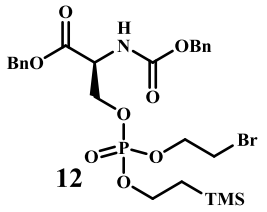
2-Bromoethyl dichlorophosphate (10). The following procedure is a modification of previously published work.[140] Phosphorus (V) oxychloride (5.00 g, 3.06 ml, 32.6 mmol) was dissolved in 12ml of dry DCM. The solution was vigorously stirred under a nitrogen atmosphere while 2-bromoethanol (2.00 ml, 21.7 mol) was added over a period of ~30 minutes at room temperature. The reaction was stirred overnight. The next day the reaction was heated to 40 ^{o}C at 7mbar to remove the excess phosphorus oxychloride and the product was then purified by distillation. (b. p. 89^{o}C @ 7mbar). The product was obtained as a clear, colorless liquid. (2.44g, 46%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.57 (2H, dt, *J*=17.3 Hz, 10.5 Hz), 3.60 (2H, td, *J*=10.5 Hz, 1.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 69.9 (d, J_{CP}=14.0 Hz), 27.3 (d, J_{CP}=17.2); ^{31}P NMR (500 MHz, $CDCl_3$) δ 7.3.



11

2-Trimethylsilyl dichlorophosphite (11). The following procedure is a modification of previously published work.[128] Phosphorus trichloride (6.50 ml, 74.5 mmol) was dissolved in 60 ml of dry diethyl ether in flask equipped with an addition funnel. The mixture was cooled down to -40^{o}C and 2-timethylsilylethanol (7.12 ml, 49.7 mmol) in 60 ml of diethyl ether was added

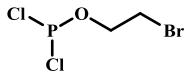
over 2 hours. The reaction was stirred for 1 more hour after completion of the addition. The reaction was then warmed to 0°C and the solvent was removed under high vacuum. The crude oil was then purified by vacuum distillation (b.p. 39 °C at high vacuum). The product was obtained as a clear, colorless liquid (6.37 g, 29.1 mmol, 58%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.36 (2H, q, *J*=8.3 Hz), 1.12 (2H, t, *J*=8.5 Hz), 0.07 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 67.5 (d, J_{CP}=9.7 Hz), 19.3 (d, J_{CP}=2.6 Hz), -1.5; ^{31}P NMR (500 MHz, $CDCl_3$) δ 177, 167 (impurity).



12

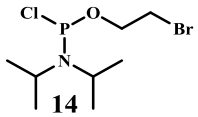
Benzyl *N*-((benzyloxy)carbonyl)-*O*-((2-bromoethoxy)(2-(trimethylsilyl)ethoxy)phosphoryl)-*L*-serinate (12). 11 (2.16 g, 9.86 mmol) was dissolved in 50ml of dry THF and the solution was cooled to -78 °C. DIPEA (4.30 ml, 24.6 mmol) was then added followed by a dropwise addition of Z-Ser-OBn (3.24 g, 9.86 mmol, in 10ml of dry THF). The reaction was stirred at the same temperature of 1 hour after which 2-bromoethanol (903 µl, 9.86 mmol) was added via syringe. The reaction was stirred at -78 °C for 30 more minutes and then overnight at room temperature. TLC showed full conversion; 150 ml of diethyl ether was then added to the reaction and the solution was filtered through a glass frit. The solvent was removed under reduced pressure and the resulting oil was dissolved in 30 ml of DCM. The solution was cooled to 0°C and 1.90 ml of 70% tert-butyl hydroperoxide solution in water was added. The reaction was stirred vigorously at the same temperature for 1 hour. The excess peroxide was then quenched with $P(OMe)_3$ (700 µl) and the solution was warmed to room temperature. The water was then removed using $MgSO_4$ and the solvent was removed under reduced pressure. The crude oil was then chromatographed using 30% EtOAc/Hexane as eluent, yielding the product as a clear and colorless oil (4.30 g,

65.0 mmol, 70%, mixture of diastereomers). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31-7.35 (10H, 2 br s), 5.83-5.89 (1H, 2 d, *J*=8.2 Hz), 5.30 (DCM), 5.21 (2H, m), 5.12 (2H, m), 4.56-4.63 (1H, m), 4.35-4.48 (2H, m), 4.04-4.20 (4H, m), 3.90 (EtOAc), 3.41-3.56 (2H, 2 t, *J*=5.6 Hz), 2.04 (EtOAc), 1.25 (EtOAc), 0.99-1.11 (2H, 2 t, *J*=8.7 Hz), -0.01-0.02 (9H, 2 s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1 (EtOAc), 168.8, 155.8, 136.0, 134.9, 128.0-128.6 (aromatic region), 67.8, 67.3-67.4 (m), 67.2, 66.8 (d, J_{CP}=5.3 Hz), 62.8, 60.4 (EtOAc), 54.5, 35.9, 29.4 (d, J_{CP}=7.5 Hz), 21.0 (EtOAc), 19.5 (d, J_{CP}=5.3 Hz), 14.2 (EtOAc), -1.5.



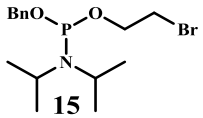
13

2-Bromoethyldichlorophopshite (13). Phosphorus (III) chloride (40.0 ml, 457 mmol) was charged into a 100 ml two-neck flask; the material was stirred and cooled to -25 ^{o}C (1,3-dichlorobenzene/dry ice). 2-bromoethanol (20.0 ml, 218 mmol) was then added via an addition funnel over the course of 2.5 hours while the reaction was vigorously stirred. The reaction was then stirred overnight as the cooling bath expired. The next day the addition funnel was replaced with a short-path distillation assembly as the reaction was kept under positive nitrogen pressure. The excess phosphorus (III) chloride was removed by heating the reaction to 40 ^{o}C at 7mbar. The mixture distilled at 3-6 mbar with a distillate coming over at 75-90 ^{o}C. The product was obtained as a clear, colorless liquid and was stored in a solvent pot at -20 ^{o}C (47.6 g, 211 mmol. 97%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.47-4.51 (2H, m), 3.56 (2H, t, *J*=6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 66.8 (d, J_{CP}=10.2 Hz), 28.4 (d, J_{CP}=3.1 Hz); ^{31}P NMR (500 MHz, $CDCl_3$) δ 178.8.



14

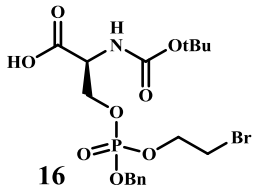
N,N-diisopropyl-2-bromoethyl-chlorophosphoramidite (14). 2-bromoethyldichlorophosphite (47.0 g, 210 mmol) was dissolved in 700 ml of dry THF and the mixture was cooled to -78 ^{o}C. To an addition funnel were added diisopropylamine (29.4 ml, 210 mmol), triethylamine (30.9 ml, 220 mmol), and 100 ml of dry THF. The addition funnel solution was then added to the reaction vessel over 1.5 hours. Following the addition, the reaction was stired at -78 ^{o}C for 1 more hour. The cooling bath was then removed and the reaction was stirred overnight at room temperature. The next day the reaction was filtered through a glass frit and the solvent was removed under reduced pressure in anhydrous conditions. The resulting cloudy orange oil was distilled under high vacuum with a distillate coming over at 90 ^{o}C. The distillate was found to be the product, which was obtained as a colorless, slightly cloudy liquid (45.3 g, 166 mmol, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.04-4.18 (2H, m) , 3.78 (2H, dq, *J*=18Hz, 6.8Hz), 3.53 (2H, q, *J*=6.3Hz), 1.25 (12H, dd, *J*=26Hz, 6.5Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 65.4 (d, J_{CP}=17Hz), 46.0 (d, J_{CP}=13Hz), 30.5 (d, J_{CP}=7.2Hz), 23.9, 23.2 (d, J_{CP}=12Hz); ^{31}P NMR (500 MHz, $CDCl_3$) δ 181.3.



15

N,N-diisopropyl-benzyl-2-bromoethylphosphoramidite (15). N,N-diisopropyl-2-bromoethyl-chlorophosphoramidite (6.64 g, 22.8 mmol) and diisopropylethylamine (4.40 ml, 25.1 mmol) were dissolved in 125 ml of dry THF and cooled to 0 ^{o}C. Benzyl alcohol (2.60 ml, 25.1 mmol) was then added via a syringe and the solution was stirred for 30 minutes at the same temperature. The reaction was then diluted to 750 ml with diethyl ether and the solids were filtered using a glass frit. The solvent was removed under reduced pressure yielding a cloudy, yellow oil. The crude was then chromatographed using 8% ethyl acetate in hexane +2% triethylamine as eluent

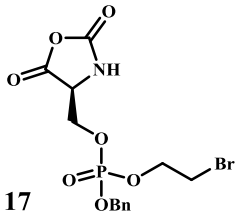
(R_f = 0.4). The desired fractions were combined and concentrated under reduced pressure and the volatiles were removed under vacuum, yielding a clear and colorless oil (7.53 g, 208 mmol, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25-7.37 (5H, m), 4.66-4.79 (2H, m), 3.85-4.0 (2H, m), 3.62-3.70 (2H, m), 3.48 (2H, t, *J*=6.7Hz), 1.20 (12H, t, *J*=7.2Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.2, 128.3, 127.3, 127.0, 65.5 (d, J_{CP}=18Hz), 63.5 (d, J_{CP}=18Hz), 43.1 (d, J_{CP}=12Hz), 31.6 (d, J_{CP}=6.8Hz), 24.6 (t, J_{CP}=7.7Hz); ^{31}P NMR (500 MHz, $CDCl_3$) δ 148.7.



16

O-((benzyloxy)(2-bromoethoxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serine (16). Boc-Ser-OH (1.37 g, 6.68 mmol) were dissolved in 12 ml of dry THF. N-methylmorpholine (808 µl, 7.34 mmol) were then added via a syringe followed by tert-butyldimethylsilyl chloride (1.11 g, 7.34 mmol), which resulted in the immediate formation of a while solid. The suspension was then stirred for 40 minutes at room temperature. Phosphoramidite **15** (3.15 g, 8.68 mmol) was then added via a syringe followed by 40 ml of 3-4% 1H-tetrazole in dry acetonitrile. The reaction clarified within one minute and was stirred for another 2 hours at room temperature. The mixture was then cooled to 0^{o}C using an ice bath and was treated with a 70% solution of *t*-butyl hydroperoxide (1.72 ml, 13.4 mmol). The ice bath was removed 5 minutes after the addition was complete and the reaction was stirred for another 30 minutes. The excess peroxide was then quenched with a saturated aqueous solution of sodium thiosulfate. Diethyl ether was then added until the total volume equaled 150 ml and was extracted with 5% $NaHCO_3$ (3x30 ml). The aqueous layer was acidified with concentrated HCl to a pH of 1-2 and was extracted with DCM (3x20 ml). The combined DCM washes were then dried over $MgSO_4$ and concentrated under

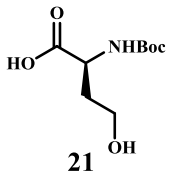
reduced pressure. The volatiles were removed under high vacuum yielding the product as a viscous, clear, colorless oil (3.06 g, 6.35 mmol, 95%).$[\alpha]_D^{21}$= +10.0^o (c = 10 mg/ml MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (1H, br s), 7.30-7.40 (5H, br s), 5.60 (1H, m), 5.06-5.16 (2H, m), 4.44-4.55 (2H, m), 4.21-4.33 (3H, m), 3.44 (2H, t, *J*=6.1Hz), 1.45 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (d, J_{CP}=5.4Hz), 155.5 (d, J_{CP}=5.3Hz), 135.06 (d, J_{CP}=6.6Hz), 128.9 (d, J_{CP}=5.6Hz), 128.7 (d, J_{CP}=2.6Hz), 128.2, 80.5, 70.2 (t, J_{CP}=4.7Hz), 68.1, 67.2 (d, J_{CP}=5.2Hz), 53.6, 29.2, 28.3; ^{31}P NMR (500 MHz, $CDCl_3$) δ -1.50; HRMS-ESI (*m/z*) [M – H] Calcd. For $C_{17}H_{25}BrNO_8P$, 480.0423; found 480.0437.



17

2-Bromoethylbenzylphosphoserine N-carboxyanhydride (17). O-((benzyloxy)(2-bromoethoxy)phosphoryl)-N-(tert-butoxycarbonyl)-L-serine (2.40 g, 4.98 mmol) was dissolved in 30 ml of dry THF. The solution was treated with distilled N-methylmorpholine (550 µl, 4.98 mmol) followed by phosgene (5.00 ml, 9.95 mmol, 20% solution in toluene). A white solid was formed immediately following the addition. The solution was stirred and heated to 50 ^{o}C overnight. The next day the volatiles and the excess phosgene were removed under high vacuum. **Note that phosgene is extremely toxic and an extended evacuation time of at least 4 hours is essential to remove all phosgene traces.** The phosgene in the vacuum traps was then quenched with 30% aqueous ammonia. The residue in the flask was then redissolved in 30 ml of dry THF and the solids were removed using a glass fritted funnel. The solvent was removed under reduced pressure and the resulting orange oil was chromatographed on vacuum-dried silica[141] using 1:1 Hex/EtOAc as eluent, no additional precautions were taken. The NCA is visualized by TLC

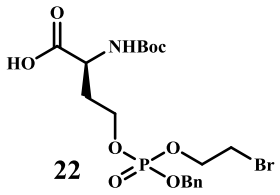
using both UV and ninhydrin stain (there are 2 spots which elute with an Rf of ~0.5, the desired product is the second spot). The fractions containing the NCA were then combined and concentrated under reduced pressure. The resulting clear, pale yellow oil was then brought into a glove box and passed through a silica plug using dry THF as eluent. The solvent is then removed under vacuum yielding the product as a clear, pale yellow oil (1.28 g, 3.14 mmol, 63%). The product was stored in a -28 ^{o}C freezer over the course of 4 weeks with no noticeable decrease in quality. $[\alpha]_D^{23}$ = -10.1 (c = 10 mg/ml in MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (1H, br s), 7.37-7.41 (5H, m), 5.09 (2H, dt, *J*=12Hz), 4.22-4.46 (5H, overlapping region), 3.43-3.46 (2H, m), 1.45 (trace starting material); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.0, 151.7 (d, J_{CP}=3.4Hz), 134.8 (dd, J_{CP}=8.1Hz, 6.0Hz), 129.2, 128.8, 128.3 (d, J_{CP}=4.7Hz), 70.6 (dd, J_{CP}=13Hz, 6.0Hz), 67.5 (dd, J_{CP}=15Hz, 5.5Hz), 65.2 (dd, J_{CP}=12Hz, 5.2Hz), 58.2 (d, J_{CP}=6.4Hz), 29.3 (d, J_{CP}=8.0 Hz), 28.3 (impurity); ^{31}P NMR (500 MHz, $CDCl_3$) δ -1.44; HRMS-ESI (*m/z*) [M – H] Calcd. For $C_{13}H_{15}BrNO_7P$, 405.9691; found 405.9736. FTIR (THF): 1796, 1862 cm^{-1}.



21

(*tert*-butoxycarbonyl)-*L*-homoserine (21). The following procedure is a modification of previously published work.[142] *L*-homoserine (4.00 g, 33.6 mmol) was suspended in 34 ml of acetonitrile, to this suspension were then added 33.6 ml of 1.0 M NaOH. The resulting clear solution was then cooled to 0 ^{o}C and a solution of Boc_2O (7.69 g, 35.5 mmol) in 8 ml of acetonitrile were slowly added using an addition funnel. The reaction was then removed from the ice bath and was stirred overnight at ambient temperature. The next day the acetonitrile was

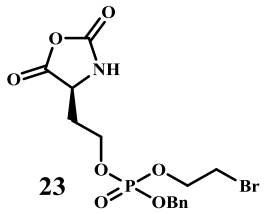
removed under vacuum. The reaction was then acidified to a pH of ~1 and NaCl was added until the solution reached saturation. The aqueous solution was then extracted with DCM (5x20 ml). The organic extracts were combined, dried over $MgSO_4$ and volatiles were removed under reduced pressure. The product was obtained as a clear colorless oil the spectral data of which matched literature.



22

***O*-((benzyloxy)(2-bromoethoxy)phosphoryl)-*N*-(*tert*-butoxycarbonyl)-*L*-homoserine (22).** **21** (2.80 g, 12.8 mmol) was dissolved in 40 ml of dry THF. N-methylmorpholine (1.47 ml, 13.4 mmol) was then added via a syringe followed by a solid addition of tert-butyldimethylsilyl chloride (2.02 g, 13.4 mmol), which resulted in the immediate formation of a while precipitate. The suspension was then stirred for 40 minutes at room temperature. Phosphoramidite **15** (4.50 ml, 14.7 mmol) was then added via a syringe followed a solid addition of pyridinium p-toluenesulfonate (6.43 g, 25.6 mmol). The suspension was clarified with the addition of acetonitrile (40 ml) and was then stirred for another 2 hours at room temperature. The mixture was cooled to 0 ^oC using an ice bath and was then treated with a 70% solution of t-butyl hydroperoxide (3.30 ml, 25.6 mmol). The ice bath was removed 5 minutes after the addition was complete and the reaction was stirred for another 30 minutes. The excess peroxide was then quenched with a saturated aqueous solution of sodium thiosulfate. Diethyl ether was added until the total volume equaled 250 ml and the resulting mixture was extracted with 5% $NaHCO_3$ (3x50 ml). The aqueous layer was acidified with concentrated HCl to a pH of 1-2 and was extracted with DCM (3x40 ml). The combined DCM washes were then dried over $MgSO_4$ and

concentrated under reduced pressure. The volatiles were removed under high vacuum yielding the product as a clear viscous, colorless oil. The oil was then further purified with column chromatography using EtOAc as an eluent. The product was obtained as a clear, colorless oil. (5.41 g, 10.9 mmol, 85%).$[\alpha]_D^{20}$= -116.0^o (c = 5 mg/ml MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (5H, m), 6.61 (1H, br s), 5.36 (1H, t, *J*=7.4 Hz), 5.11 (2H, d, *J*=8.6 Hz), 4.37-4.43 (1H, m), 4.22-4.31 (2H, m), 4.12-4.21 (2H, m), 3.46 (2H, t, *J*=6.0 Hz), 2.18-2.26 (1H, m), 2.09-2.18 (1H, m), 1.43 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$), δ 173.7, 155.6, 135.33 (d, J_{CP}=6.5 Hz), 128.8, 128.7, 128.2, 128.2, 80.3, 70.0 (d, J_{CP}=5.6 Hz), 67.0 (d, J_{CP}=5.3 Hz), 64.6, 50.4, 30.9, 29.4 (t, J_{CP}=8.3 Hz), 28.3; ^{31}P NMR (500 MHz, $CDCl_3$) δ -1.58, -1.65; HRMS-ESI (*m/z*) [M + H] Calcd. For $C_{18}H_{28}BrNO_8P$, 495.9698; found 495.9653.



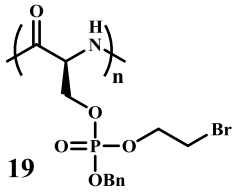
23

Benzyl (2-bromoethyl) (2-((*S*)-2,5-dioxooxazolidin-4-yl)ethyl) phosphate (23). 22 (1.20 g, 2.41 mmol) was dissolved in 15 ml of dry THF. Ghosez reagent (640 µl, 4.83 mmol) was then added via a syringe and the resulting solution was stirred overnight at ambient temperature under a nitrogen atmosphere. Volatiles were then removed using high vacuum and the resulting clear light yellow oil was brought into the glove box. The oil was then chromatographed using dry silica with 30 → 60% EtOAc in hexanes as eluent. Fractions containing the NCA were combined and concentrated under vacuum, yielding the product as a clear, colorless oil (810mg, 1.92 mmol, 80%). $[\alpha]_D^{20}$= -15.3^o (c=5 mg/ml in MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.42 (5H, m), 7.14 (1H, br s), 5.13 (2H, d, *J*=9.8 Hz), 4.37-4.42 (1H, m), 4.15-4.35 (4H, m), 3.44-3.52 (2H, m), 2.30 (1H, br s), 1.99 (1H, br s); ^{13}C NMR (125 MHz, $CDCl_3$), δ 169.7, 151.6, 135.1 (dd,

J_{CP}=5.3, 2.0 Hz), 129.16 (d, J_{CP}=5.3 Hz), 128.8 (d, J_{CP}=2.6 Hz), 128.3 (d, J_{CP}=3.0 Hz), 70.4 (t, J_{CP}=5.9 Hz), 67.3 (t, J_{CP}=5.1 Hz), 63.8 (dd, J_{CP}=5.3, 2.8 Hz), 54.7, 54.5, 32.2 (dd, J_{CP}=15.1, 6.5 Hz), 29.5 (dd, J_{CP}=12.0, 8.0 Hz); ^{31}P NMR (500 MHz, $CDCl_3$) δ -0.62, -0.73; HRMS-ESI (*m/z*) [M + H] Calcd. For $C_{14}H_{18}BrNO_7P$, 422.0004; found 421.9135. FTIR (THF): 1862, 1792 cm^{-1}.

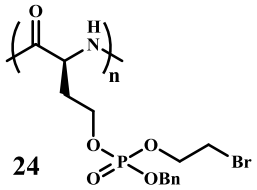
General Procedure for the synthesis of phosphate-containing polypeptides.

All polymerizations were performed in a nitrogen filled glove box using anhydrous, nitrogen purged solvents. To a solution of NCA **17** or **23** in THF (50 mg/ml) was added a solution of $Co(PMe_3)_4$ in THF (10 mg/ml) to yield a desired monomer to initiator (M:I) ratio. The reactions were let stand and were allowed to react for 6 to 12 hours, depending on M:I ratio. M:I ratios of up to 15:1 were generally complete within 6 hours, higher ratios were let run for 16 hours. Reaction progress was monitored by FTIR by following the disappearance of the NCA band at 1792 cm^{-1}. The polypeptides were isolated by precipitation into water. Polypeptide was then collected by centrifugation and the solvents removed under vacuum to yield the products as white solids (yields 89-92 %).



19

Poly(2-bromoethylbenzylphosphoserine) 19: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (1H, br s), 7.31 (5H, br s), 5.02 (2H, br s), 4.77 (1H, br s), 4.48 (1H, br s), 4.34 (1H, br s), 4.17 (2H, br s), 3.36 (2H, br s); ^{31}P NMR (500 MHz, $CDCl_3$) δ -0.69; FTIR (THF): 2338, 2116-2234, 1684 cm^{-1}.

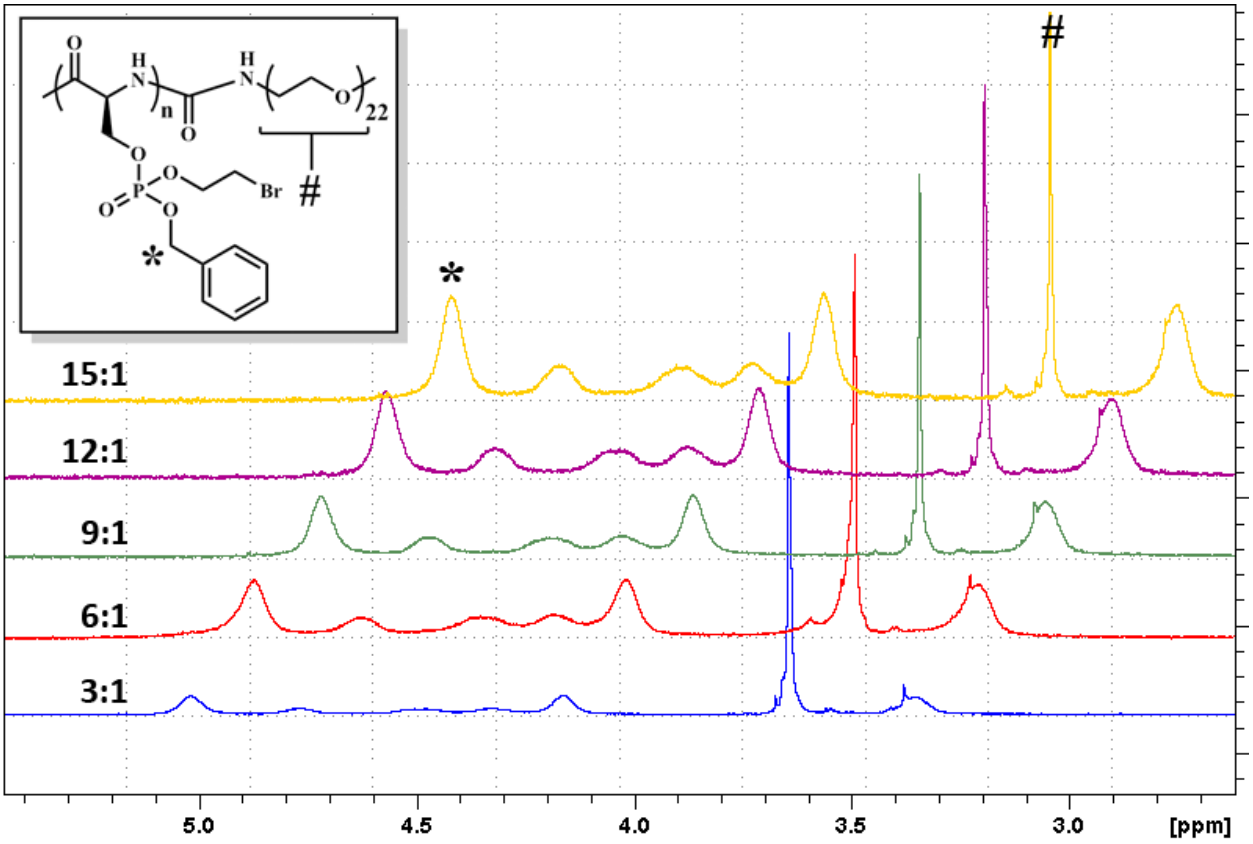


24

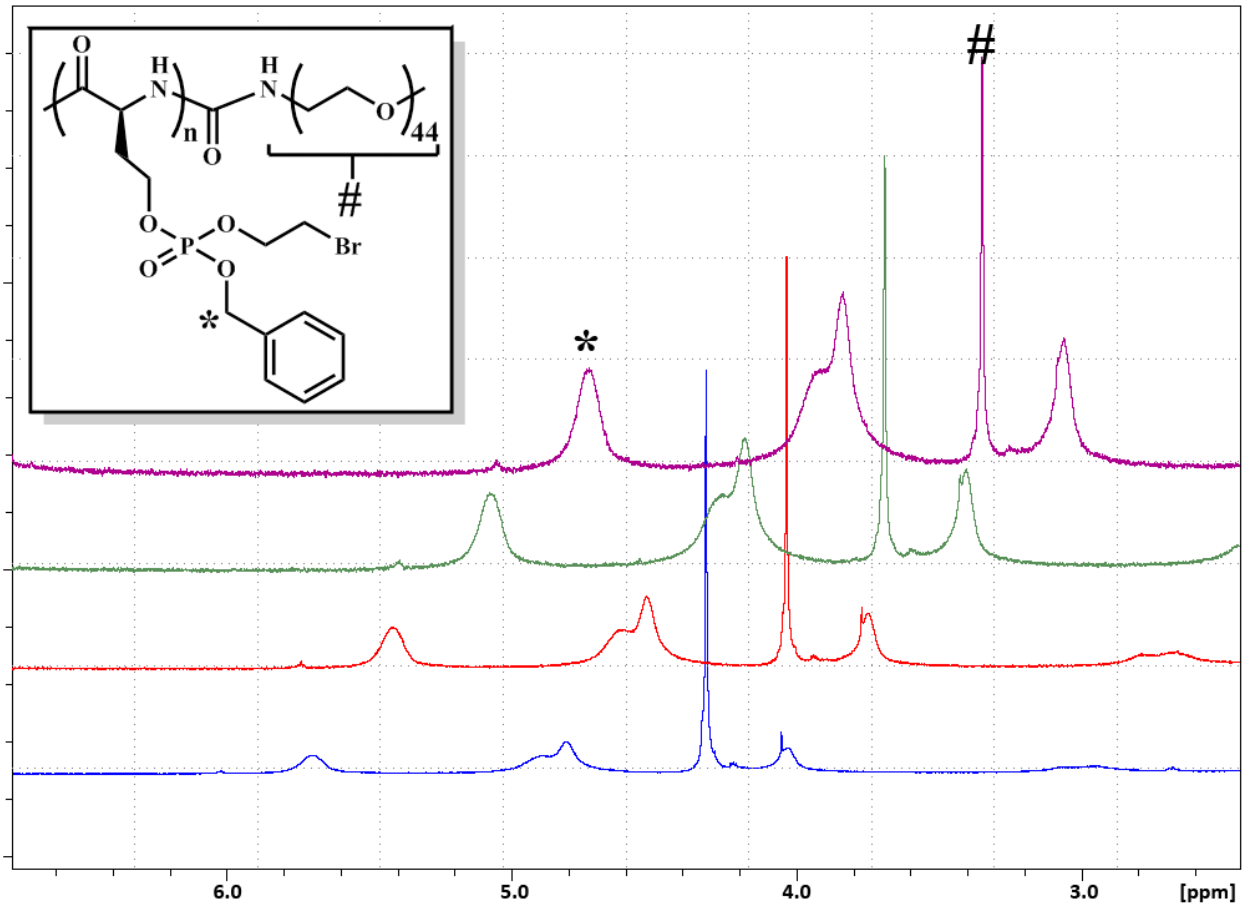
Poly(2-bromoethylbenzylphosphohomoserine) 24: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (1H, br s), 7.29 (5H, br s), 5.02 (2H, br s), 4.03-4.33 (5H, overlapping peaks), 3.36 (2H, br s), 2.07-2.46 (2H, br s); ^{31}P NMR (300 MHz, $CDCl_3$) δ -0.79; FTIR (THF): 2338, 2126-2308, 1656, 1552 cm^{-1}.

General procedure for polypeptide end-capping with poly(ethylene glycol) and chain length determination by end group analysis.

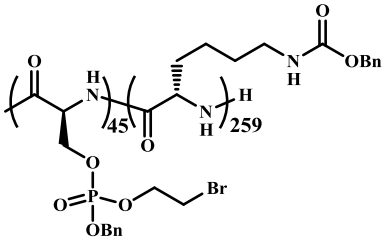
The general procedure for polymerization for phosphate-containing NCAs was first followed. Upon completion of the reaction, as confirmed by FTIR, a solution of α-methoxy-ω-isocyanoethyl-poly(ethylene glycol), PEG-NCO (MW = 1000 or 2000 Da, 3 equiv per $(PMe_3)_4Co$) in THF, was added to the polymerization reaction in a dinitrogen filled glove box. The reaction was stirred for 3 hours and then stripped of solvent under vacuum, and the resulting polymer film was suspended in 400 µl of deionized water. Use of a sonication bath was necessary to completely disperse the film. The polymer was then collected by centrifugation. Resuspension and centrifugation was repeated 2 additional times, and then water was removed from the final pellet by lyophilization. To determine polypeptide molecular weights (M_n), ^{1}H NMR spectra were obtained in deuterated chloroform. Since it has been shown that end-capping is quantitative for $(PMe_3)_4Co$ initiated NCA polymerizations when excess isocyanate is used,[87] integrations of the benzyl methylene versus the polyethylene glycol resonance at δ 3.64 were used to obtain polypeptide lengths.



^{1}H NMR spectra of PEG (1000 Da) end-capped poly(2-bromoethylphenylphospho serine **18**, . The peak areas of the polymer resonances increase relative to the constant PEG area as [M]/[I] ratio was increased.



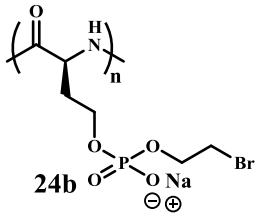
^{1}H NMR spectra of PEG (2000 Da) end-capped poly(2-bromoethylphenylphospho homoserine) **24**, . The peak areas of the polymer resonances increase relative to the constant PEG area as [M]/[I] ratio was increased.



Procedure for the synthesis of 2-bromoethylbenzylphosphoserine-containing diblock copolypeptides.

All polymerizations were performed in a nitrogen filled glove box using anhydrous, nitrogen purged solvents. To a 50 mg/ml solution of **17** in THF was added a solution of $Co(PMe_3)_4$ in THF (10 mg/ml) to yield a monomer to initiator (M:I) ratio of 9 to 1. The reaction was monitored

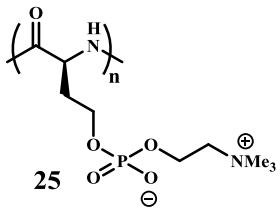
by FTIR and, upon complete consumption of the first monomer a small aliquot was removed for molecular weight analysis via PEG end-capping or GPC-LS. To the remaining active polypeptide chains was added a solution of the desired quantity of Z-Lys NCA monomer (50 mg/ml in THF, 45 eq. relative to initiator). The reaction was allowed to stand overnight and was then checked for complete monomer consumption by FTIR. A small aliquot was removed for GPC analysis and the polypeptide was isolated by precipitation of the remainder into acidic water followed by DI water (2 ml per ~10 mg of polymer). The polymer was collected by centrifugation and the residual water was removed by lyophilization to yield a white, stringy solid (90% yield). . ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (61H, br s), 7.23 (2,667H, br s, overlaps with $CDCl_3$), 5.46 (216H, br s), 5.00 (676H, br s), 4.22-4.80 (127H, overlapping signals), 4.17 (92H, br s, reference signal), 3.86 (78H, br s), 3.35 (93H, br s), 3.10 (518H, br s, CH2N comparison signal), 0.98-2.16 (2,299H, overlapping signals); FTIR (THF): 1726, 1652, 1550 cm^{-1}.



24b

Poly(2-bromoethylphosphohomoserine) Sodium Salt 24b: 24 (20 mg, DP=34) was dissolved in 2ml of 50/50 TFA/DCM. The solution was stirred at room temperature for 4 hours and then the volatiles were removed under vacuum. The resulting film was resuspended in 2 ml of 5% $NaHCO_3$, was transferred into a 2000 MWCO dialysis bag, and was dialyzed against deionized water for 3 days with twice daily water changes. The solution was then freeze dried to obtain the polymer as a white, stringy solid (16 mg, 95%). The polymer still possessed 3% of the original benzyl groups, which were completely deprotected in the subsequent step. $[\alpha]_D^{20}$= -36.0^o ^{1}H NMR

(500 MHz, *d*-TFA) δ 7.47 (0.075H, br s, residual benzyl signal), 7.42 (0.075H, br s, residual benzyl signal), 4.99 (1H, br s), 4.54 (2H, br s), 4.46 (br s), 3.81 and 3.64 (2H, br s, diastereotopic), 2.47 (1H, br s), 2.35 (1H, br s); ^{31}P NMR (300 MHz, *d*-TFA) δ -0.34.

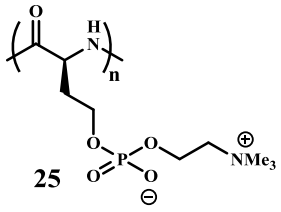


25

Poly(phosphatidylcholine homoserine) 25: The polymer can be prepared using 2 methods.

Direct Method: 24 (47 mg, DP=34) was suspended in 2 ml of aq. NMe_3 (20%). The suspension was stirred at room temperature; the staring material became completely soluble after ~1 hour. The mixture was stirred for 4 more hours and was then dialyzed against deionized water for 3 days with twice daily water changes. Sterilized Millipore water was used for sterile samples. The solution was then freeze dried to yield the product as a white, stringy solid (30mg, $1.12x10^{-4}$ mol of phosphatidylcholine side chains, 90%).

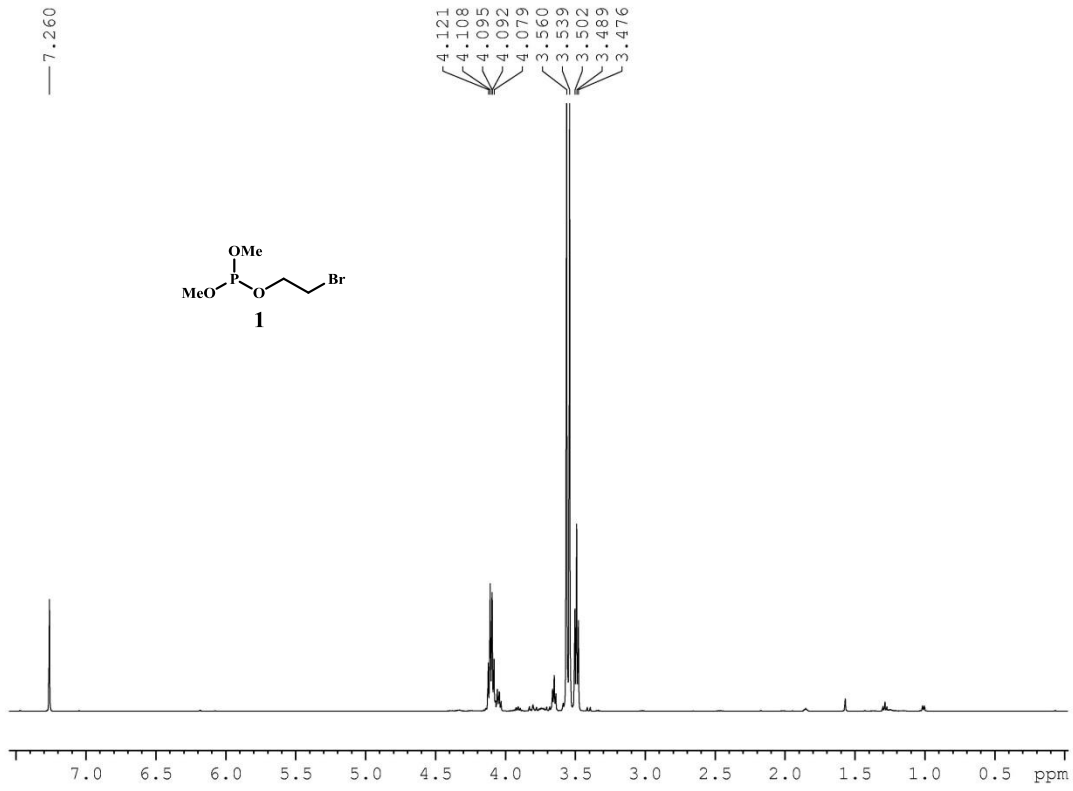
From 24b: 24b (16 mg, DP=34) was dissolved in 1 ml of aq. NMe_3 (20%). The mixture was stirred for 5 hours and was then dialyzed against deionized water for 3 days with twice daily water changes. Sterilized Millipore water was used for sterile samples. The solution was then freeze dried to yield the product as a white, stringy solid (11mg, 95%).

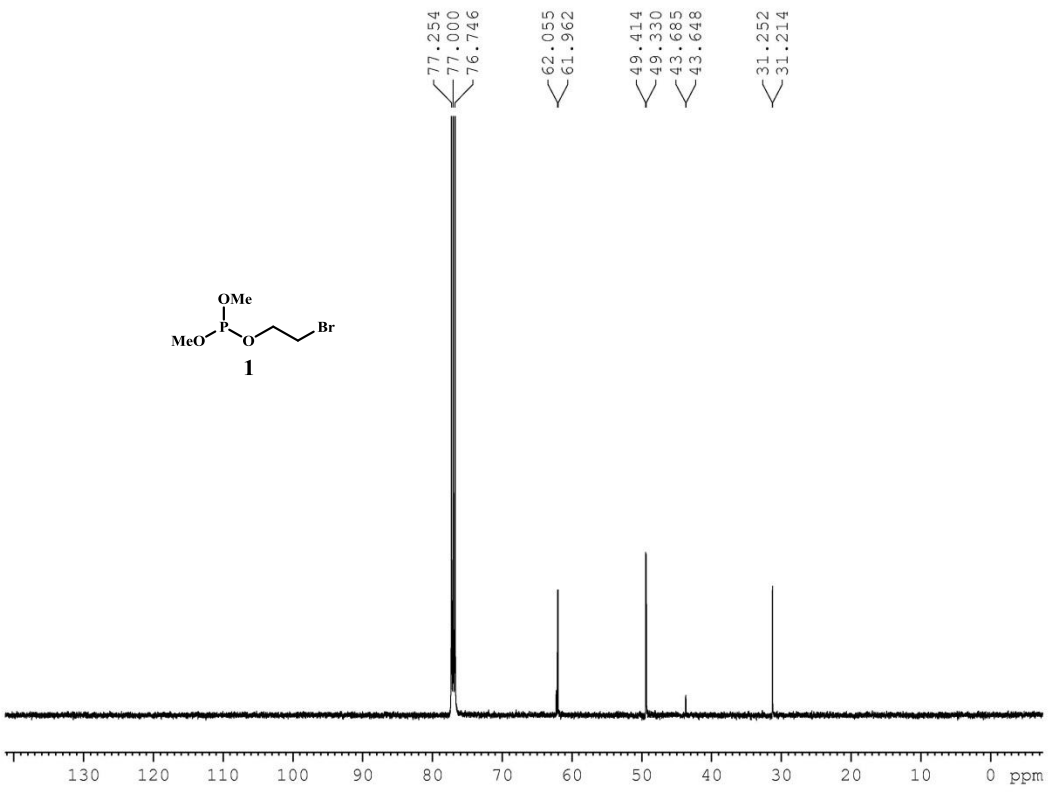


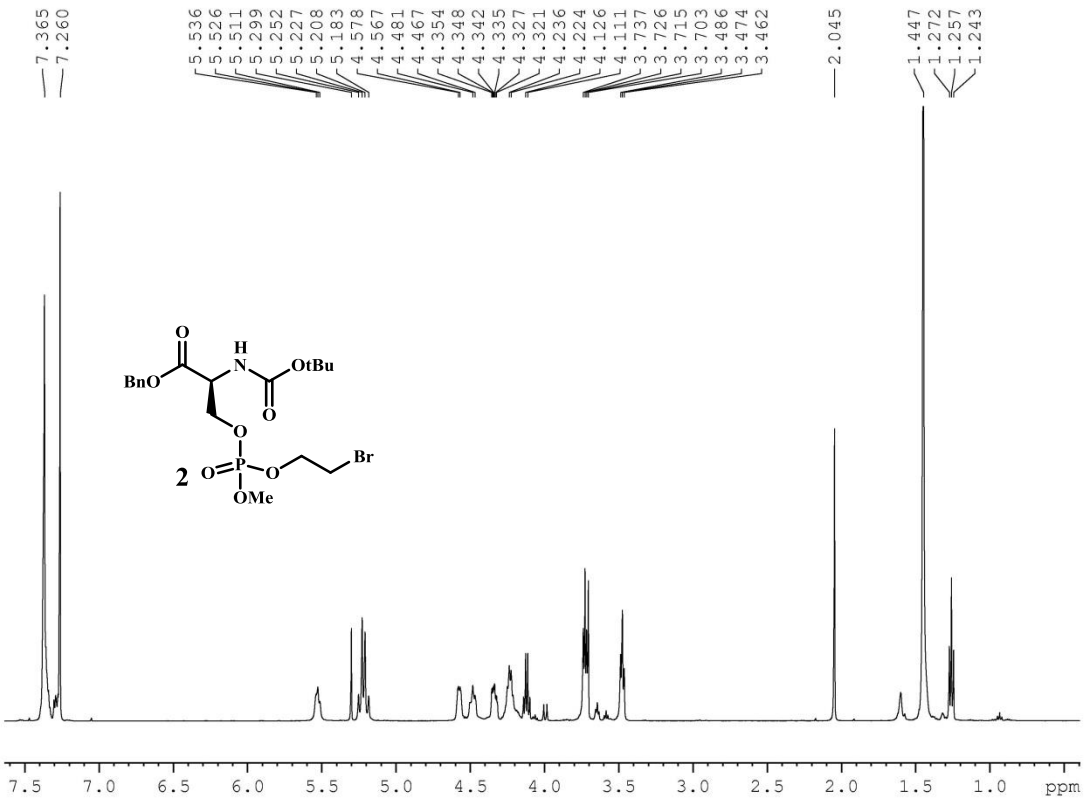
25

$[\alpha]_D^{20}$= -47.9^o (c=5 mg/ml in H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.49 (1H, br s), 4.31 (2H, br s), 4.02 (1H, br s), 3.94 (1H, br s), 3.68 (2H, br s), 3.24 (9H, br s), 2.17 (1H, br s), 2.03 (1H, br s); ^{31}P NMR (300 MHz, $CDCl_3$) δ 0.23.

4.9.3 Spectral Data



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4.236
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1.272
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O
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O
O
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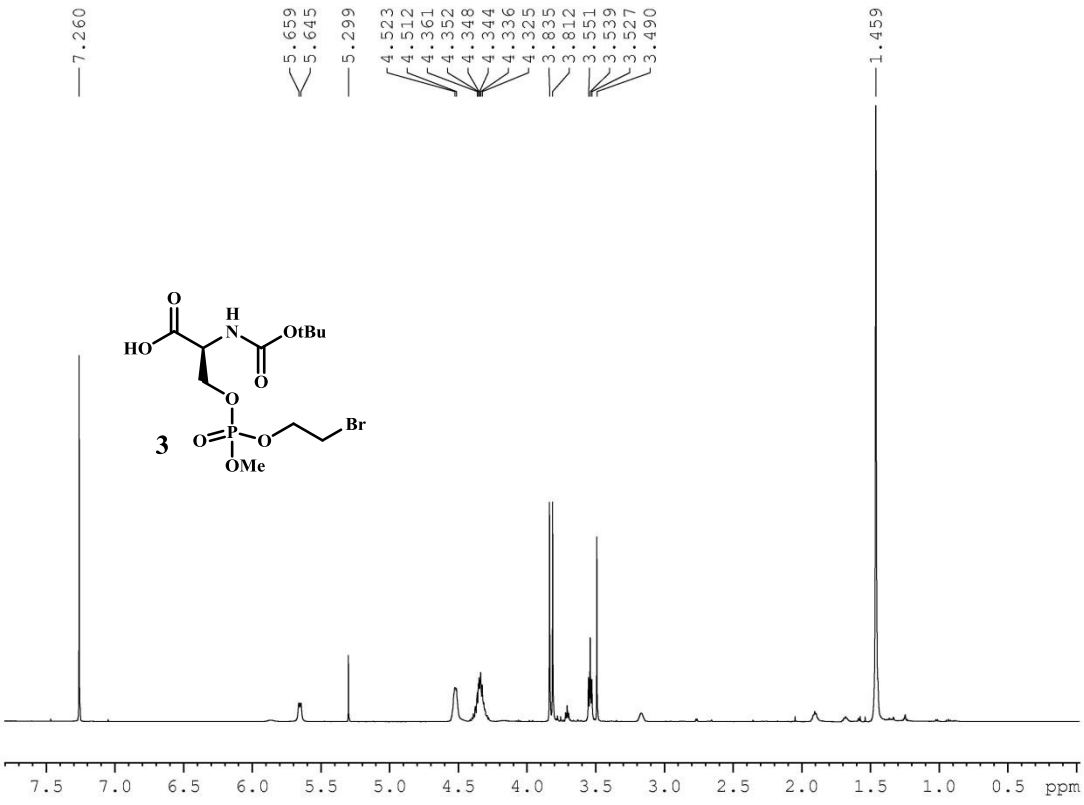
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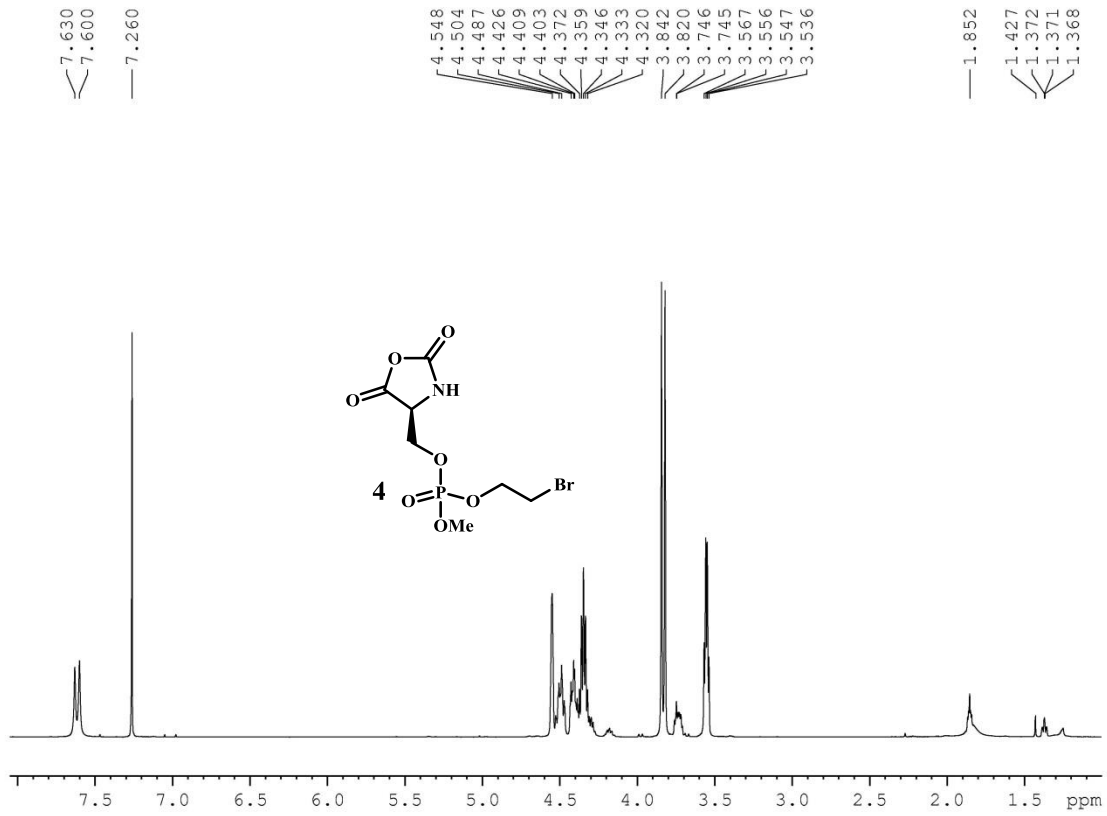
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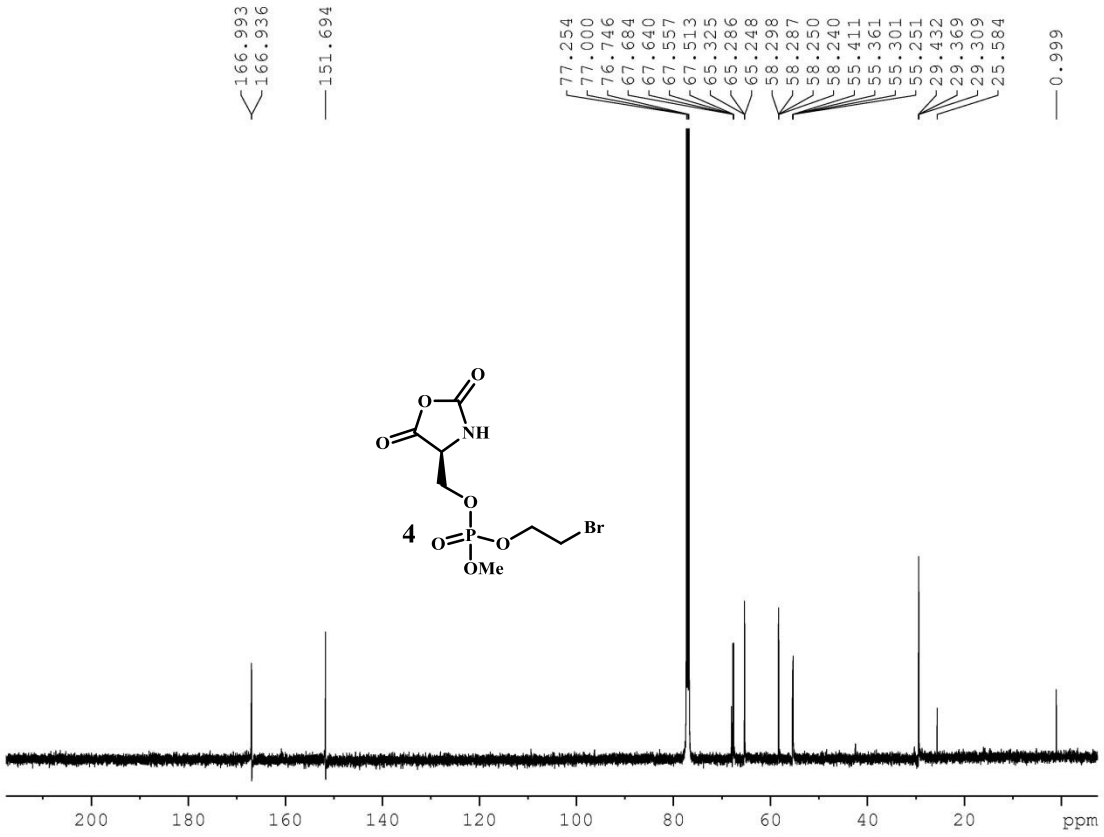
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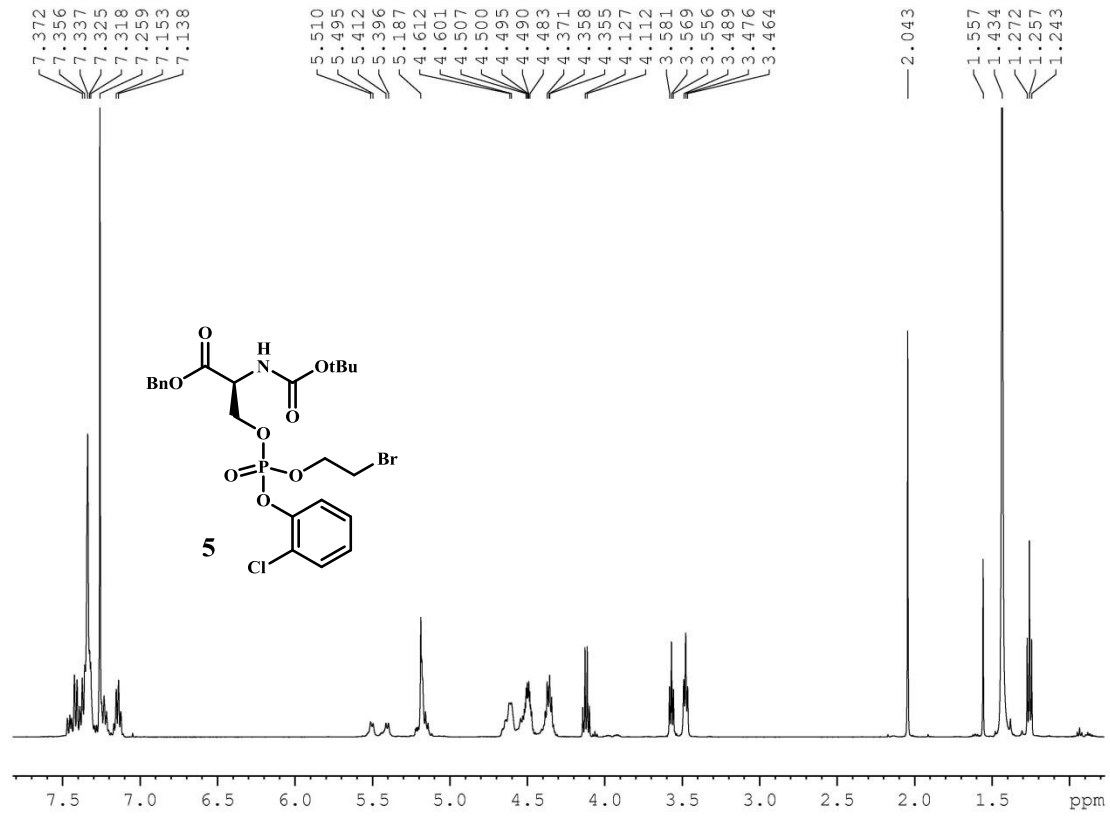
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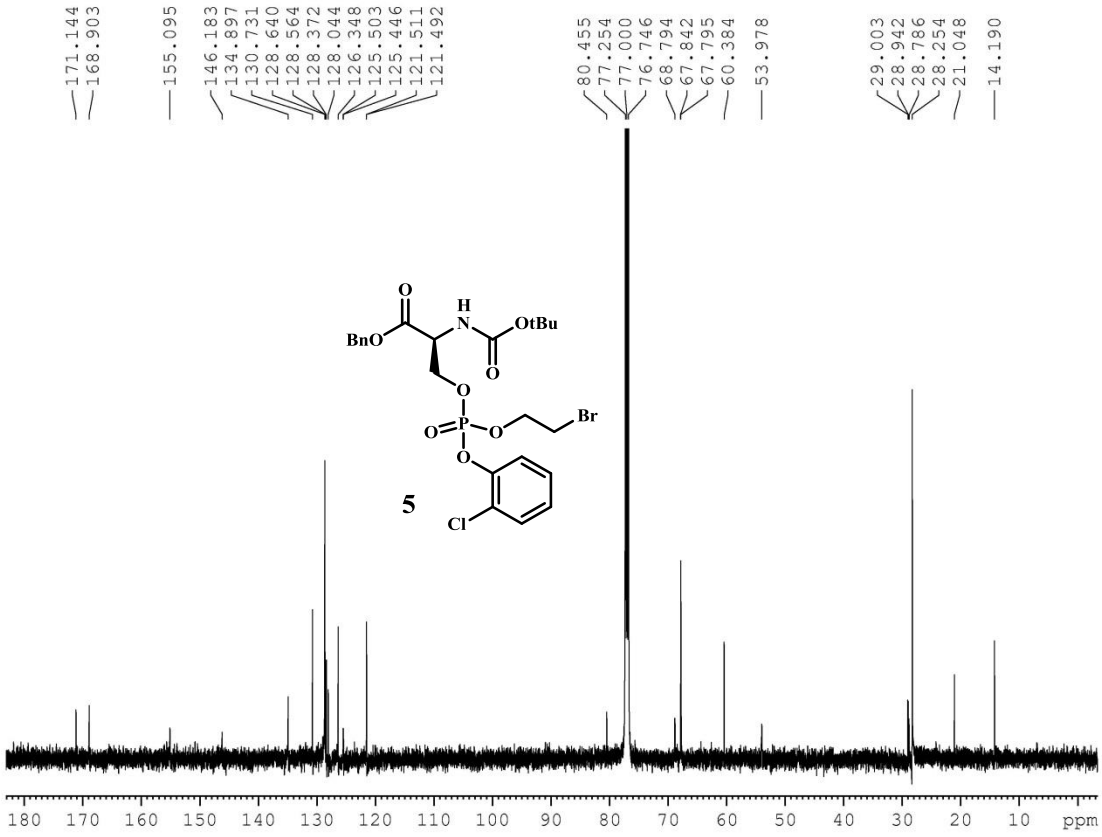
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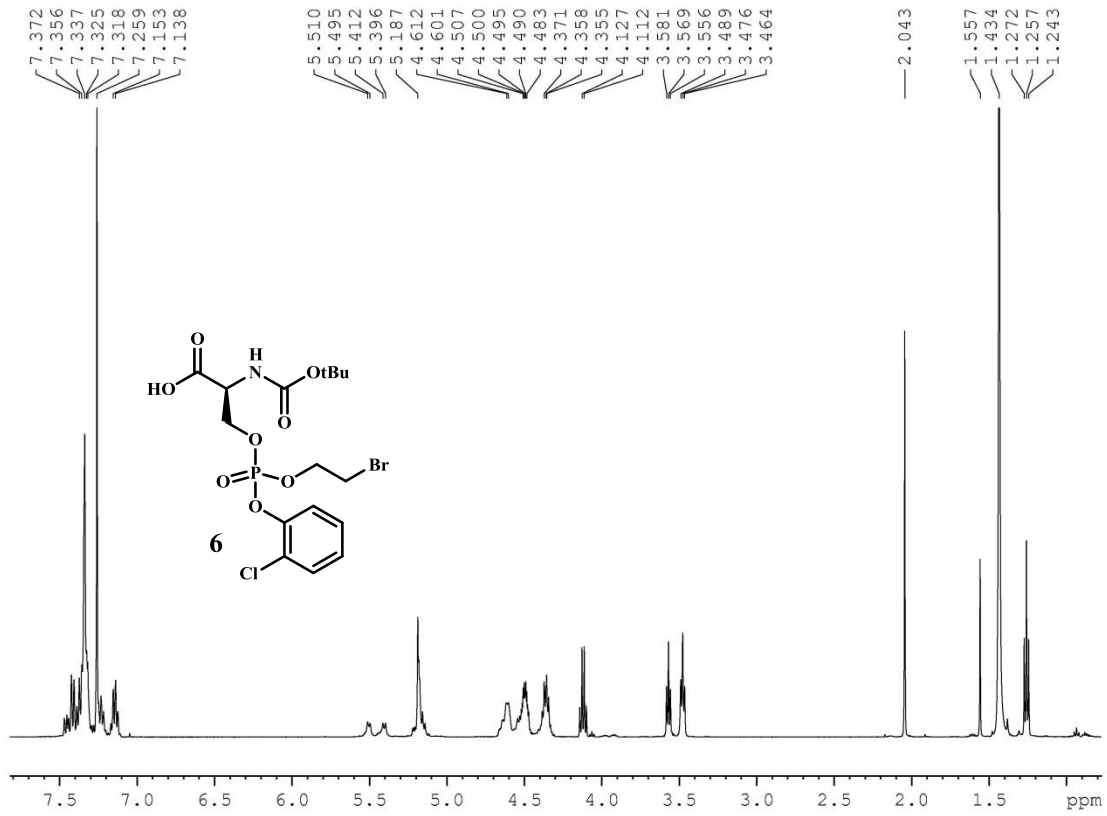
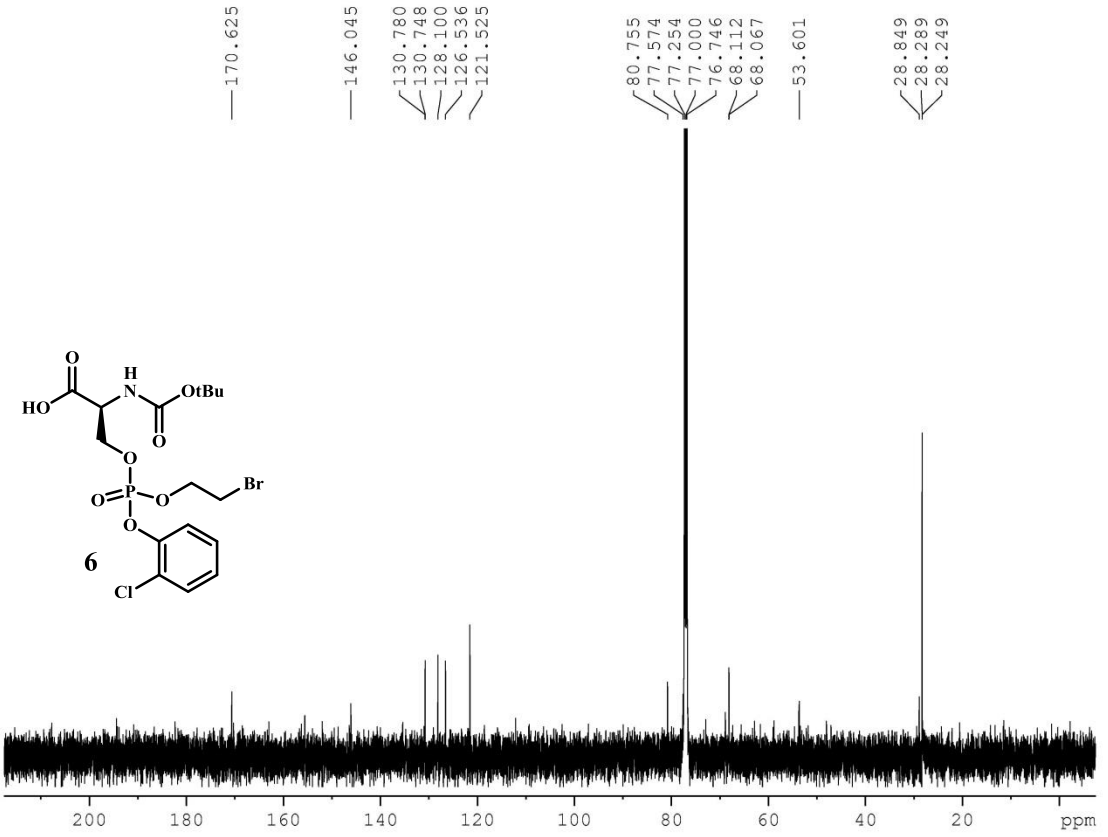
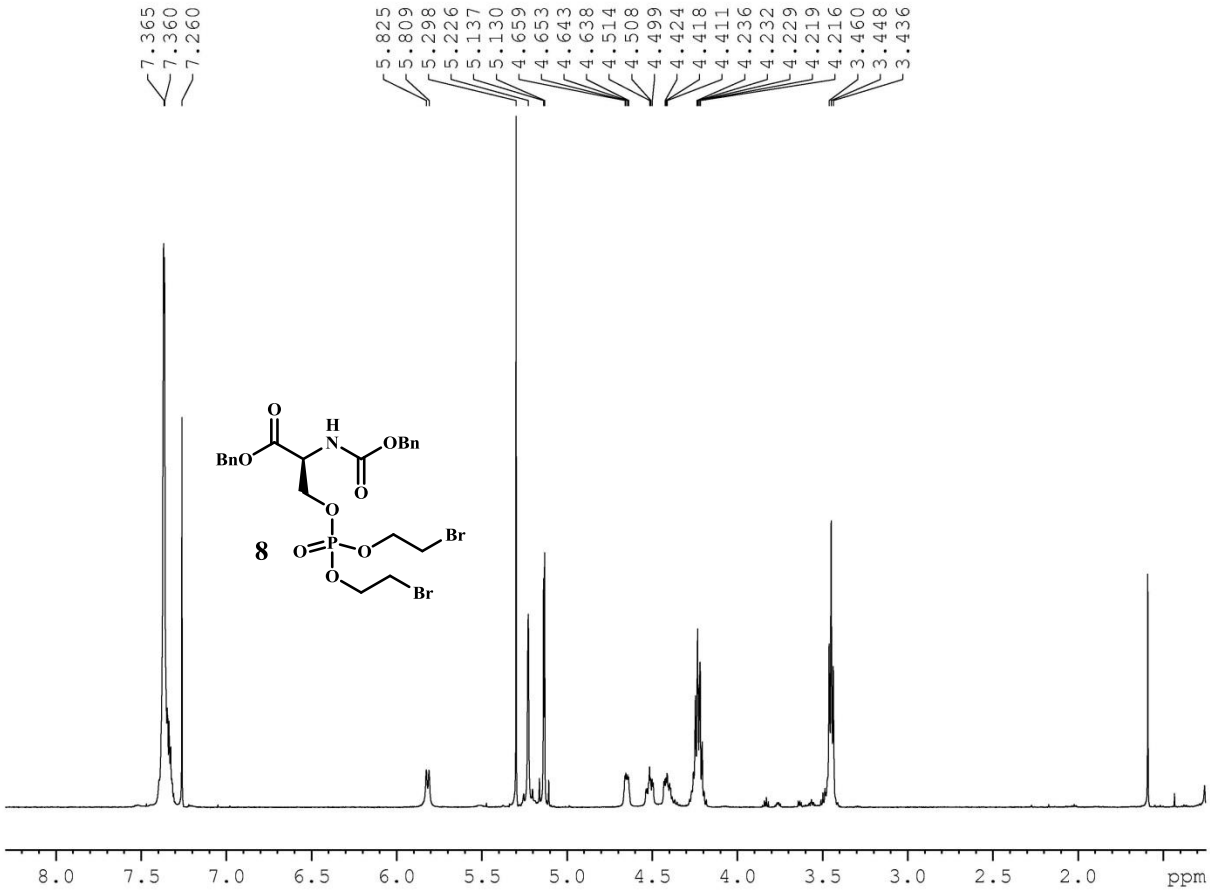
128.564
128.372
128.044
126.348
125.503
125.446
121.511
121.492
80.455
77.254
77.000
76.746
68.794
67.842
67.795
60.384
53.978
29.003
28.942
28.786
28.254
21.048
14.190
BnO
OtBu
Br
Cl
5
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


7.372
7.356
7.337
7.325
7.318
7.259
7.153
7.138
5.510
5.495
5.412
5.396
5.187
4.612
4.601
4.507
4.500
4.495
4.490
4.483
4.371
4.358
4.355
4.127
4.112
3.581
3.569
3.556
3.489
3.476
3.464
2.043
1.557
1.434
1.272
1.257
1.243
HO
H
N
OtBu
O
O
O
P
O
O
Br
O
Cl
6
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm


170.625
146.045
130.780
130.748
128.100
126.536
121.525
80.755
77.574
77.254
77.000
76.746
68.112
68.067
53.601
28.849
28.289
28.249
HO
H
N
OtBu
O
O
O
P
O
O
Br
O
Cl
6
200
180
160
140
120
100
80
60
40
20
ppm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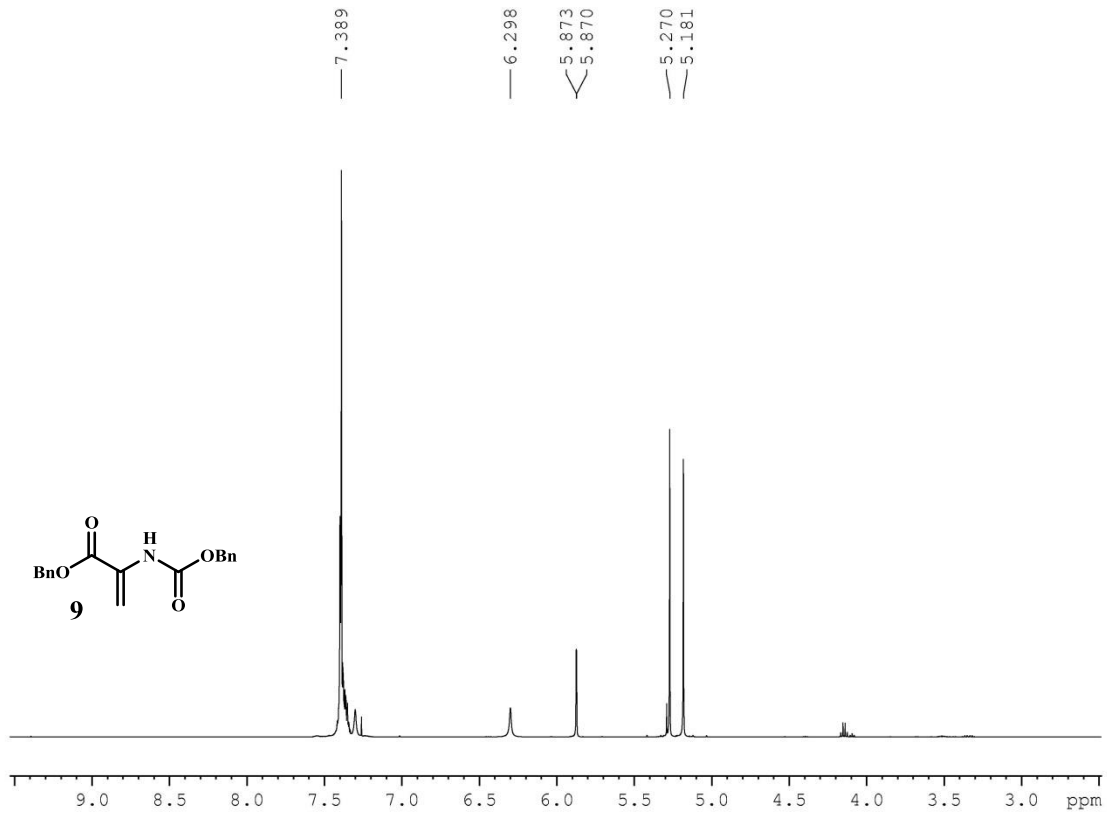
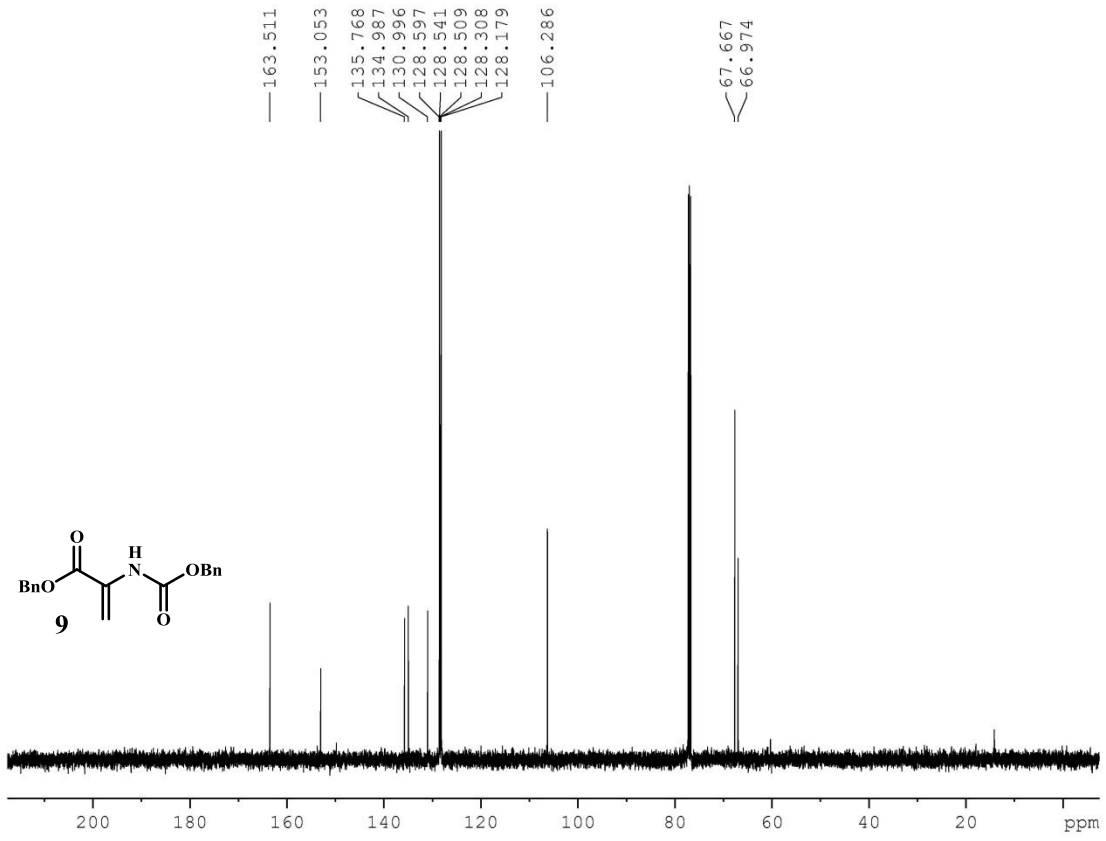
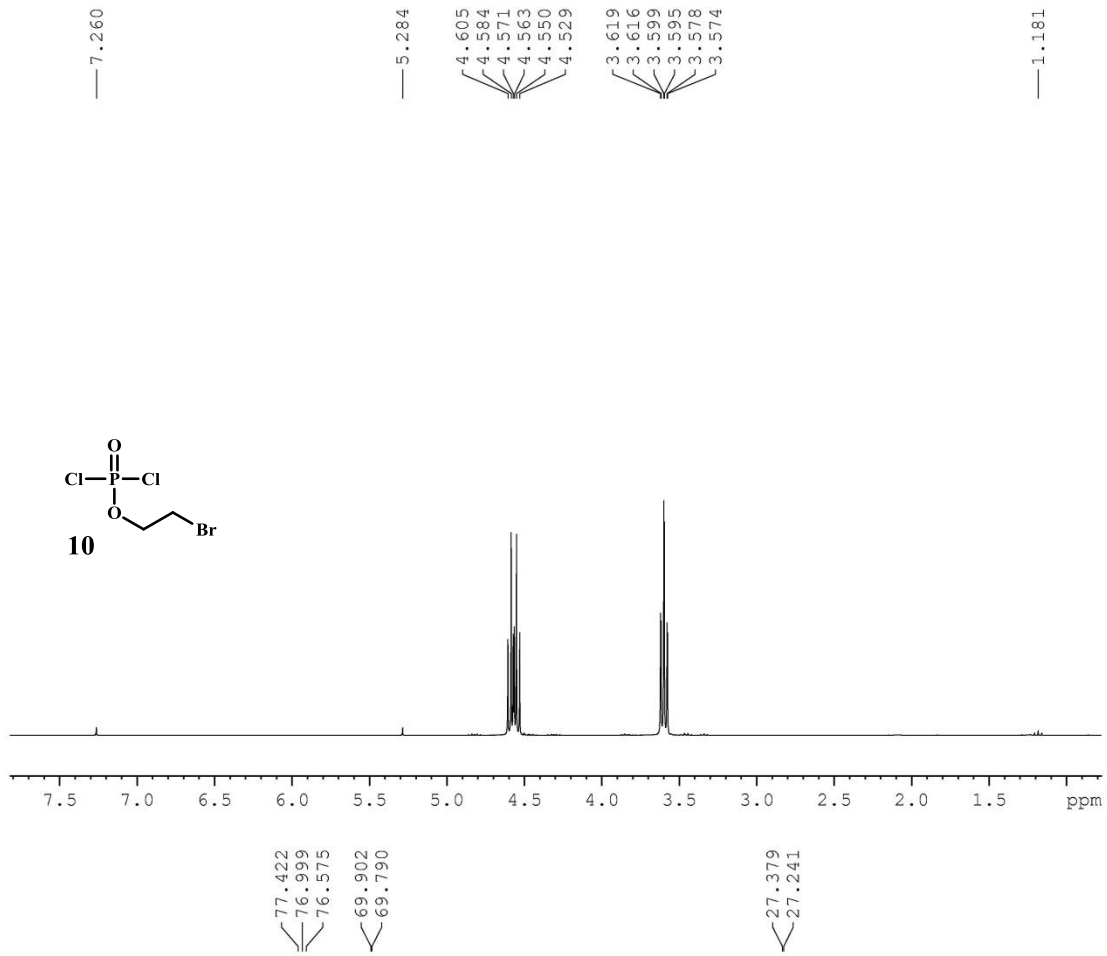
7.360
7.260
5.825
5.809
5.298
5.226
5.137
5.130
4.659
4.653
4.643
4.638
4.514
4.508
4.499
4.424
4.418
4.411
4.236
4.232
4.229
4.219
4.216
3.460
3.448
3.436
8
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm


7.389
6.298
5.873
5.870
5.270
5.181
BnO
OBn
9
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm


163.511
153.053
135.768
134.987
130.996
128.597
128.541
128.509
128.308
128.179
106.286
67.667
66.974
BnO
OBn
9
200
180
160
140
120
100
80
60
40
20
ppm


7.260
5.284
4.605
4.584
4.571
4.563
4.550
4.529
3.619
3.616
3.599
3.595
3.578
3.574
1.181
Cl
P
Cl
O
O
Br
10
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
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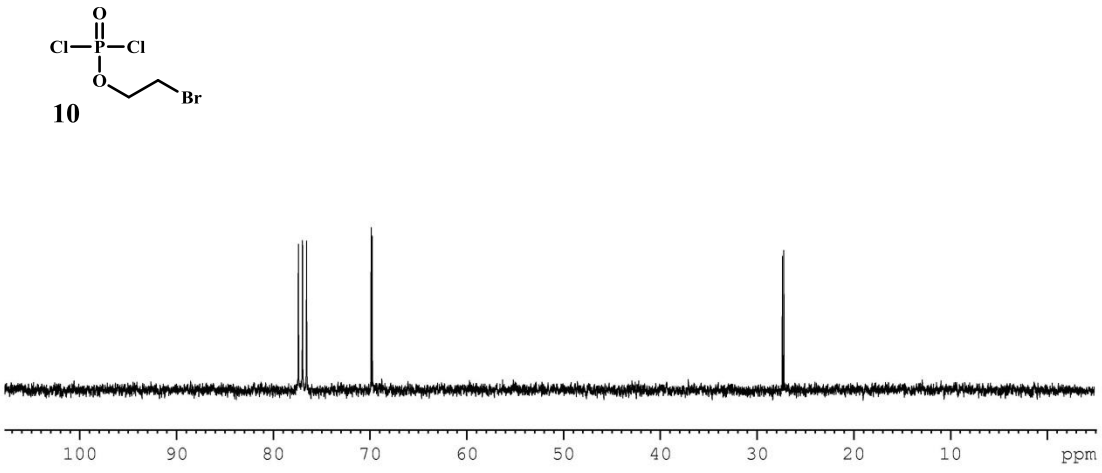
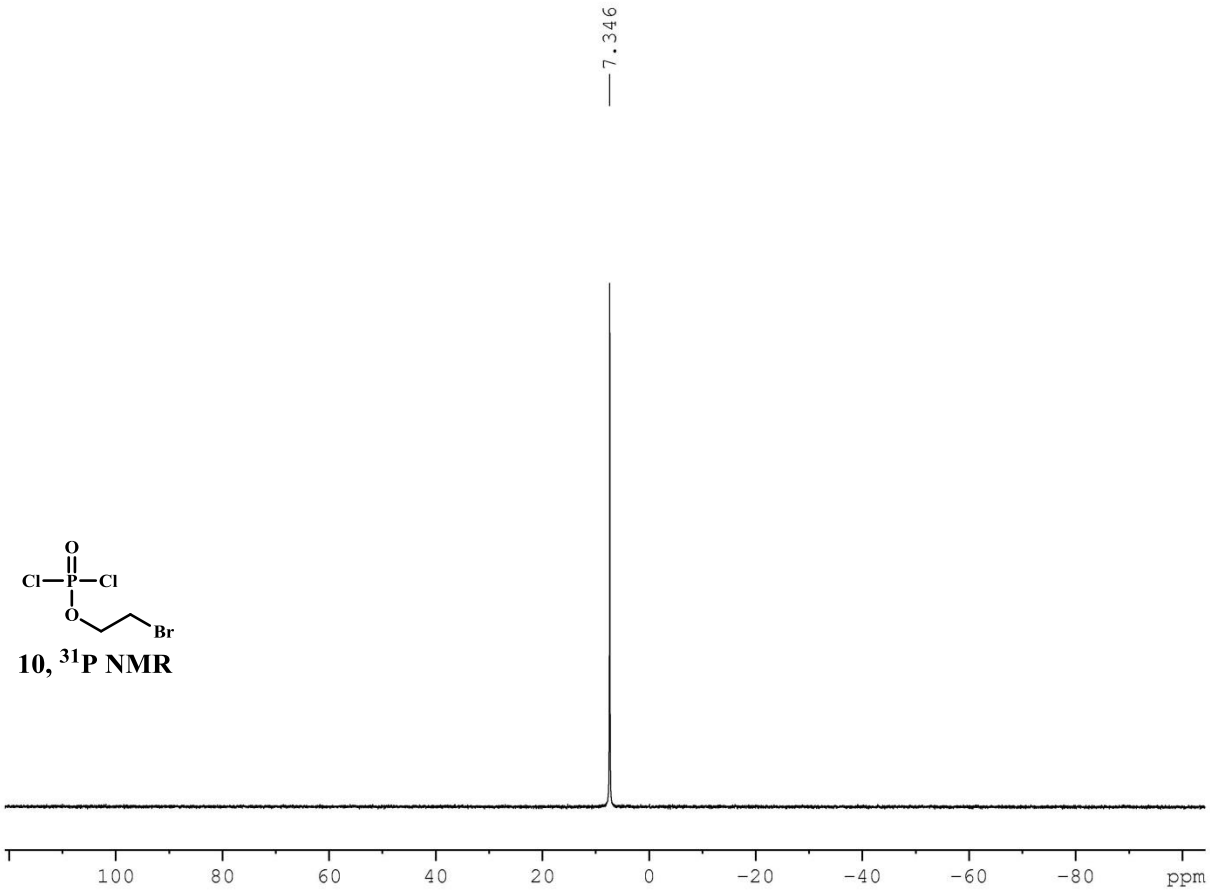
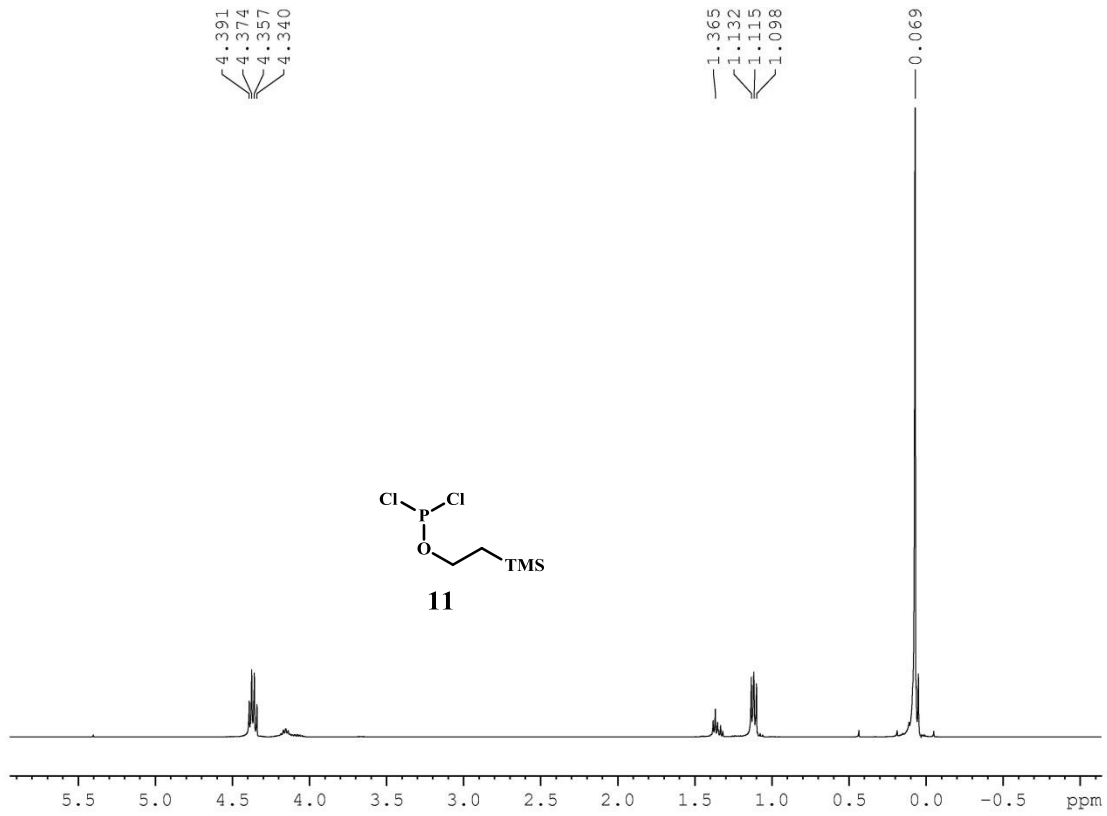
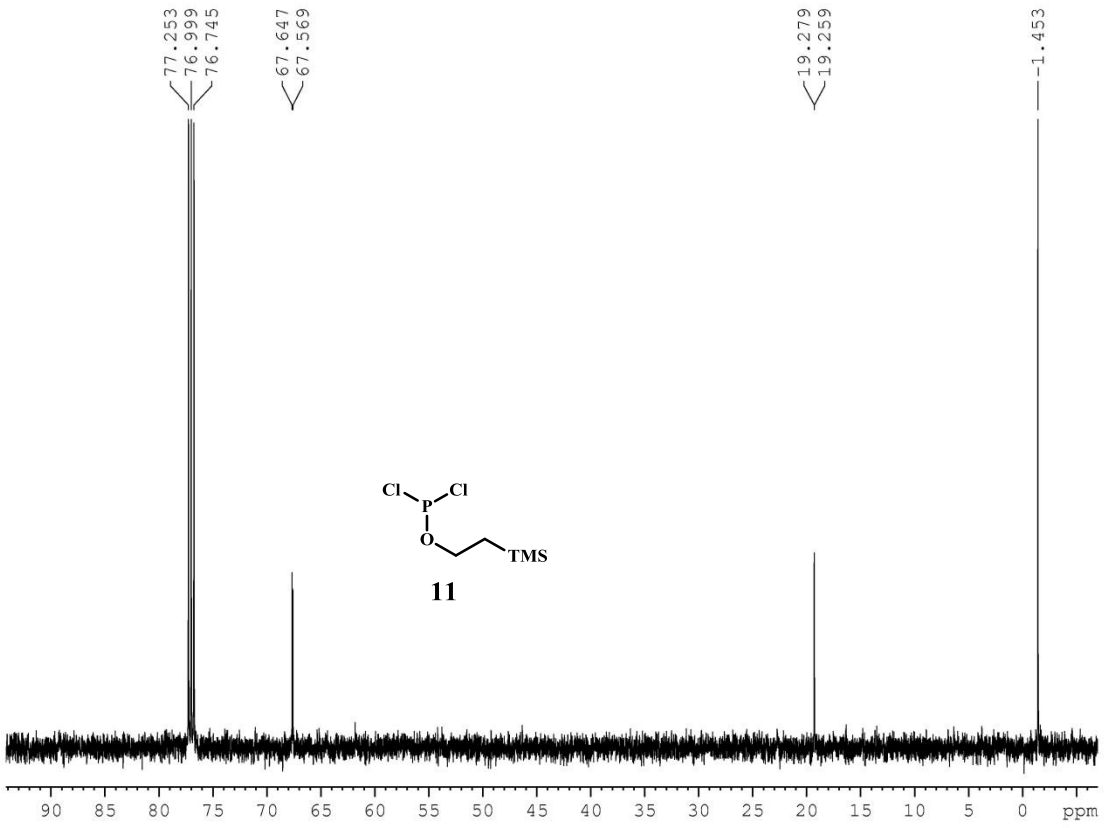
2.0
1.5
ppm
77.422
76.999
76.575
69.902
69.790
27.379
27.241

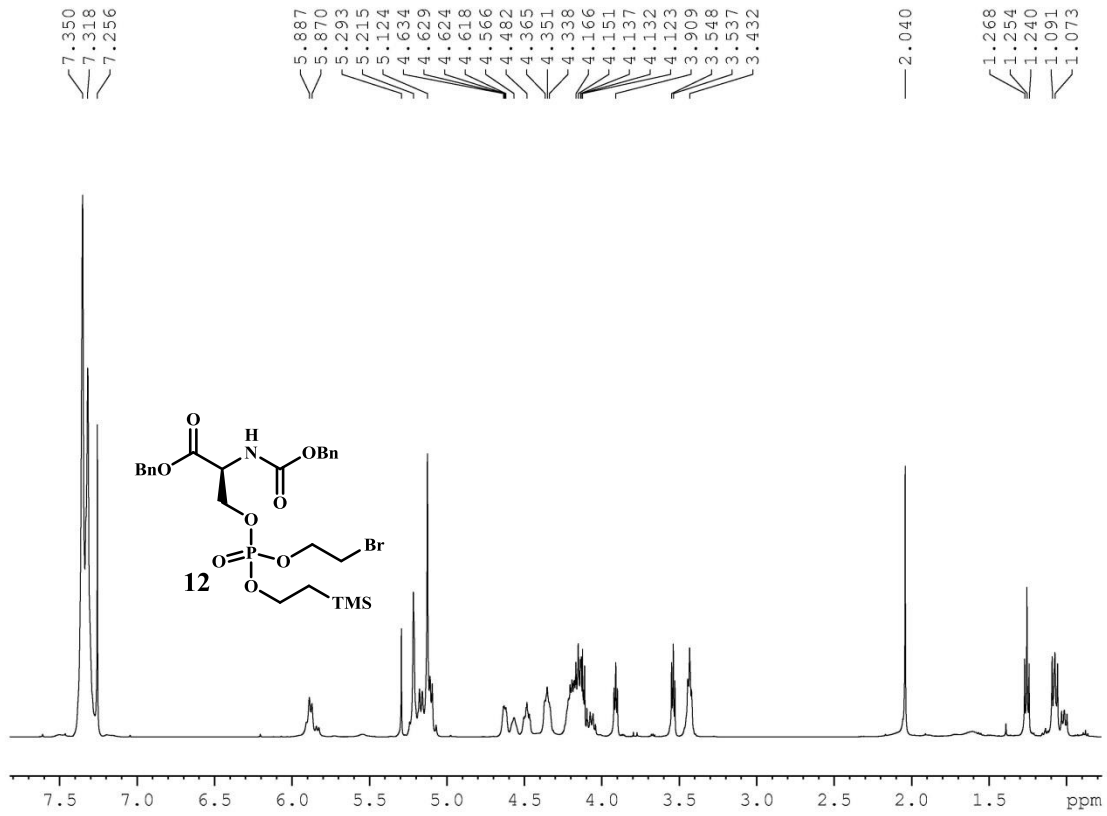

Cl
P
Cl
O
O
Br
10
100
90
80
70
60
50
40
30
20
10
ppm

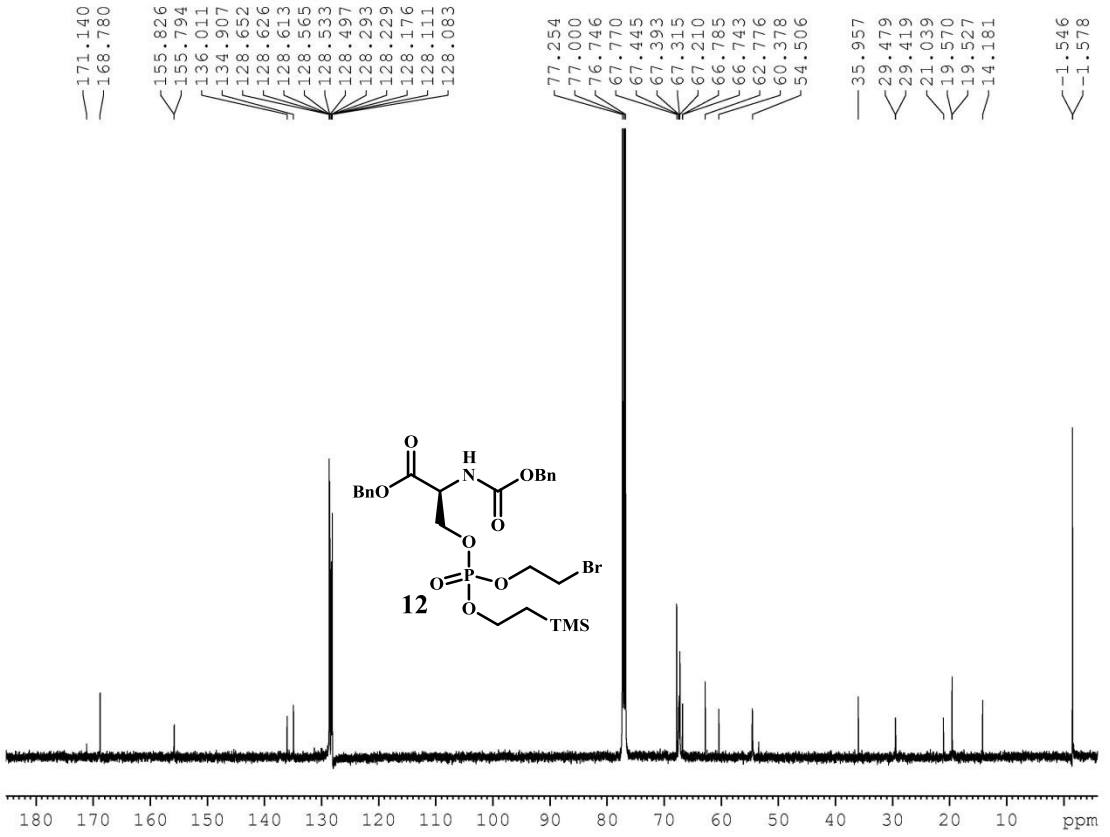


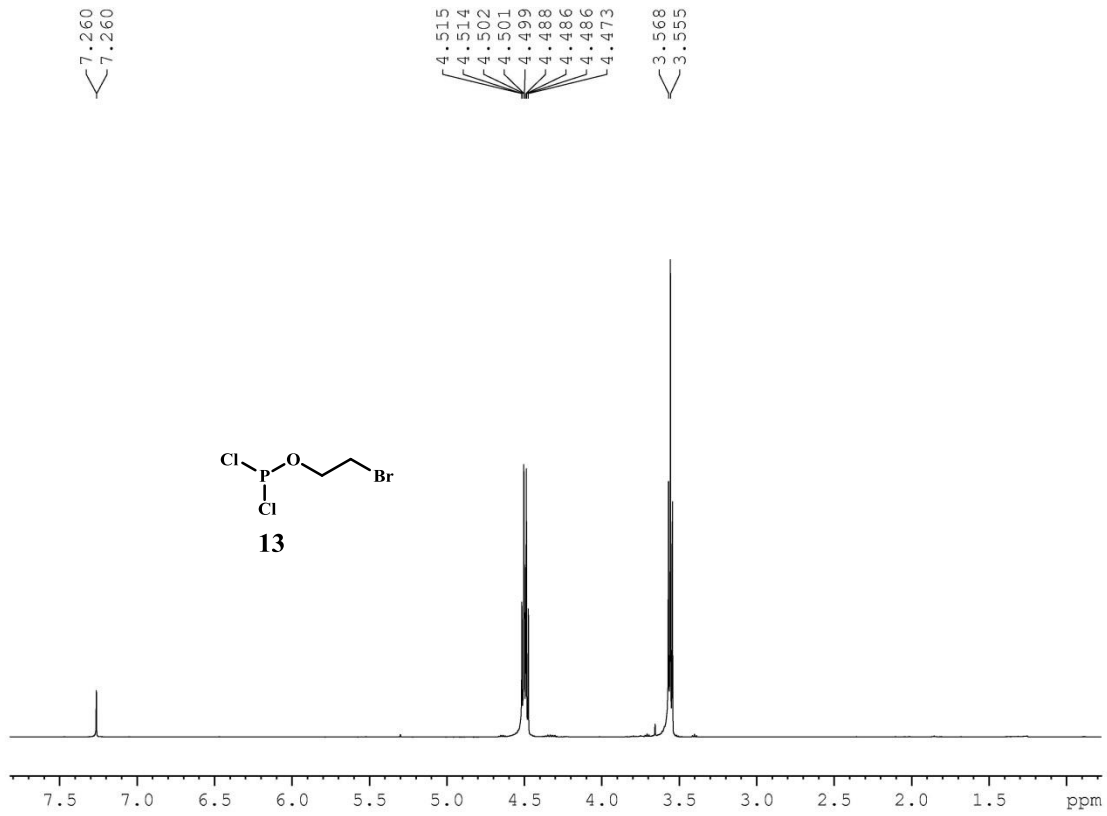
10, ^{31}P NMR

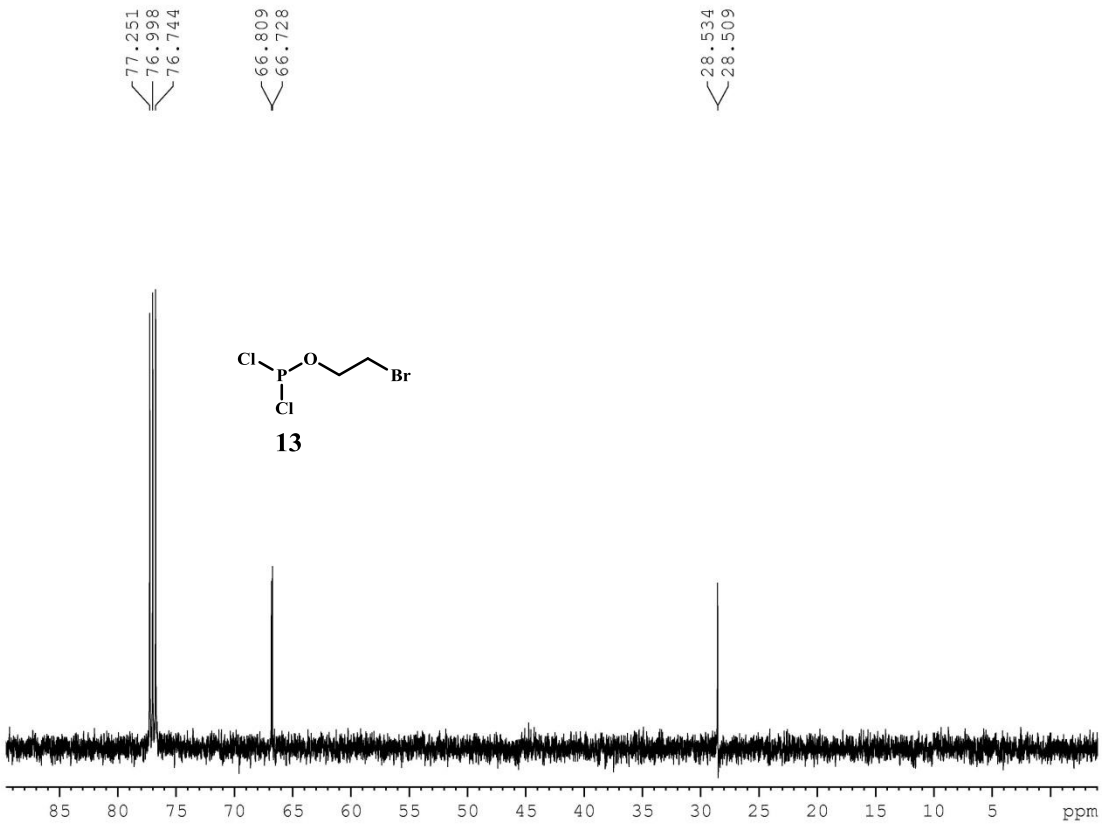

4.391
4.374
4.357
4.340
1.365
1.132
1.115
1.098
0.069
Cl
P
Cl
O
TMS
11
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ppm

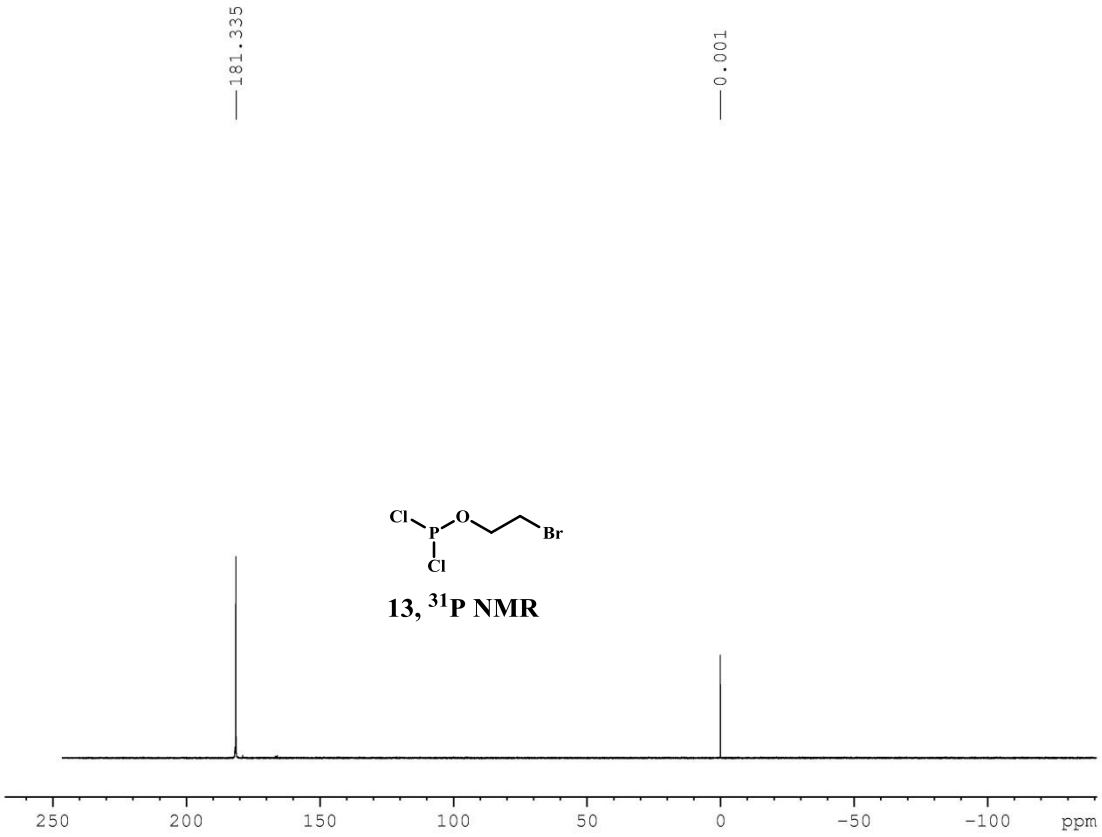

77.253
76.999
76.745
67.647
67.569
19.279
19.259
-1.453
Cl
P
Cl
O
TMS
11
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
ppm


7.350
7.318
7.256
5.887
5.870
5.293
5.215
5.124
4.634
4.629
4.624
4.618
4.566
4.482
4.365
4.351
4.338
4.166
4.151
4.137
4.132
4.123
3.909
3.548
3.537
3.432
2.040
1.268
1.254
1.240
1.091
1.073
BnO
OBn
Br
TMS
12
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm

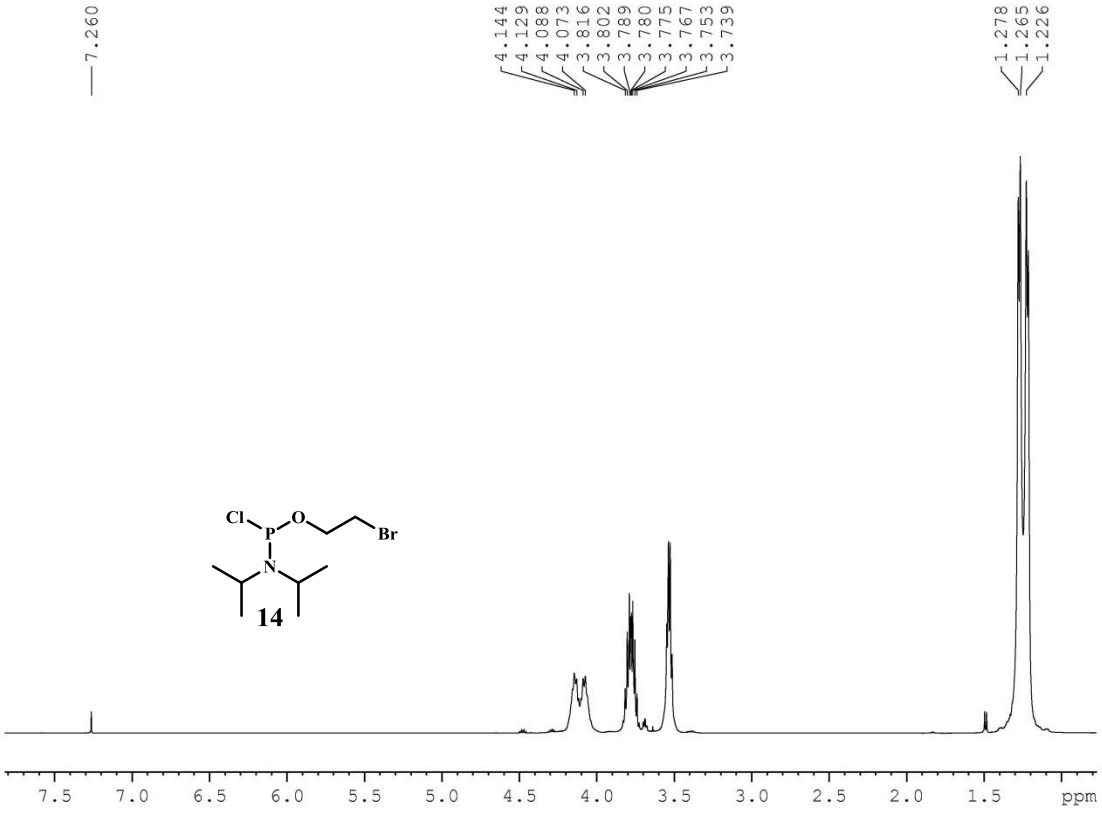

171.140
168.780
155.826
155.794
136.011
134.907
128.652
128.626
128.613
128.565
128.533
128.497
128.293
128.229
128.176
128.111
128.083
77.254
77.000
76.746
67.770
67.445
67.393
67.315
67.210
66.785
66.743
62.776
60.378
54.506
35.957
29.479
29.419
21.039
19.570
19.527
14.181
-1.546
-1.578
BnO
OBn
Br
TMS
12
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


7.260
7.260
4.515
4.514
4.502
4.501
4.499
4.488
4.486
4.486
4.473
3.568
3.555
Cl
P
O
Br
Cl
13
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm

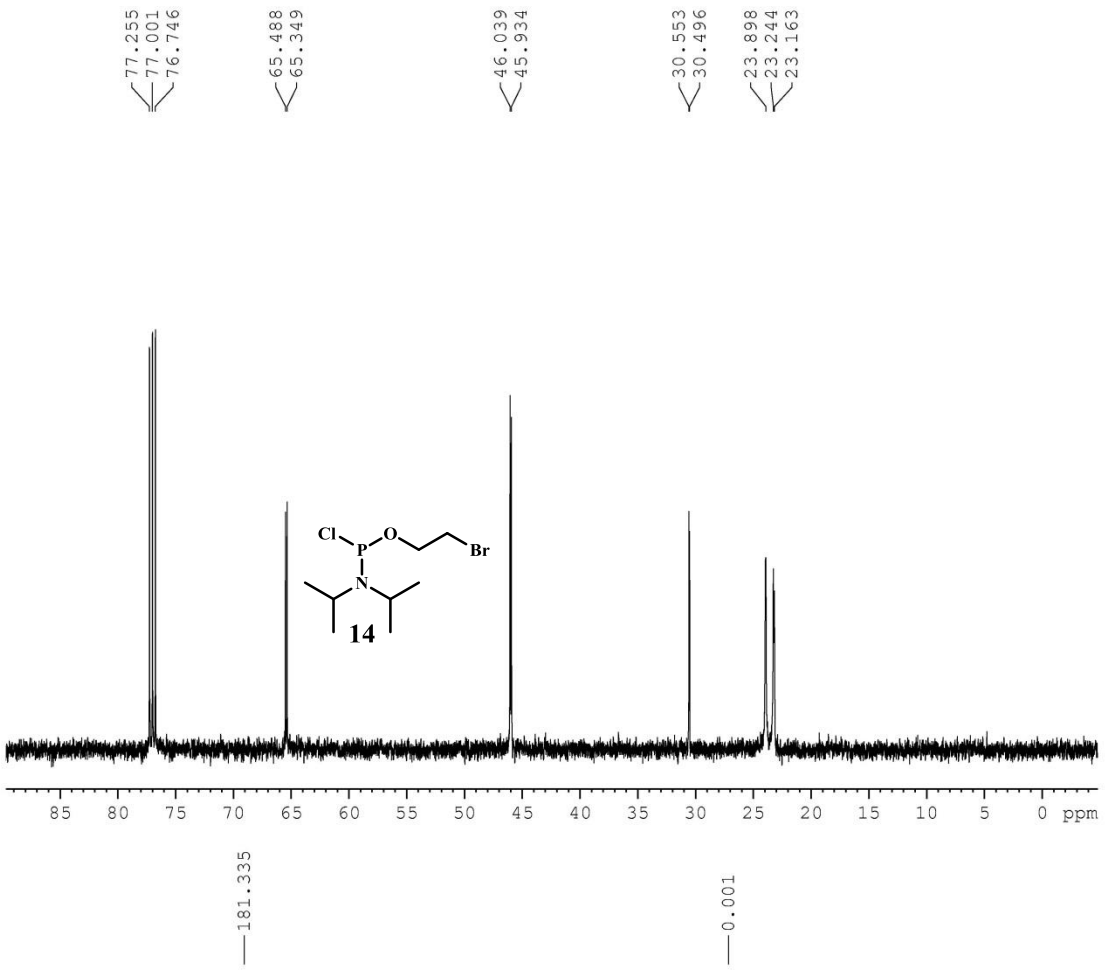

77.251
76.998
76.744
66.809
66.728
28.534
28.509
Cl
P
O
Br
Cl
13
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
ppm

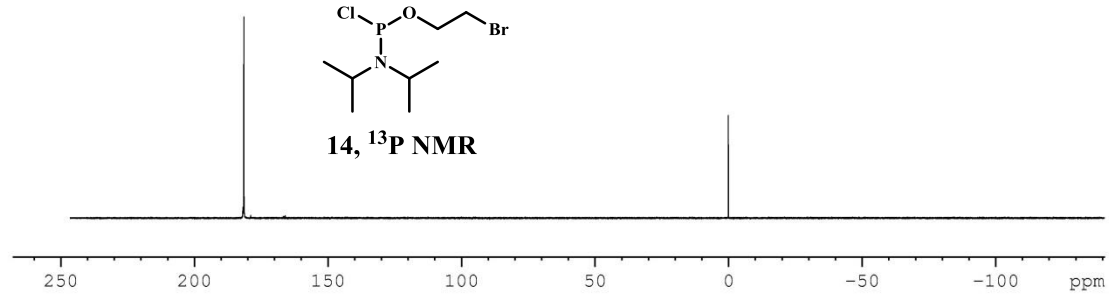

181.335
0.001
Cl
P
O
Br
Cl
250
200
150
100
50
0
-50
-100
ppm

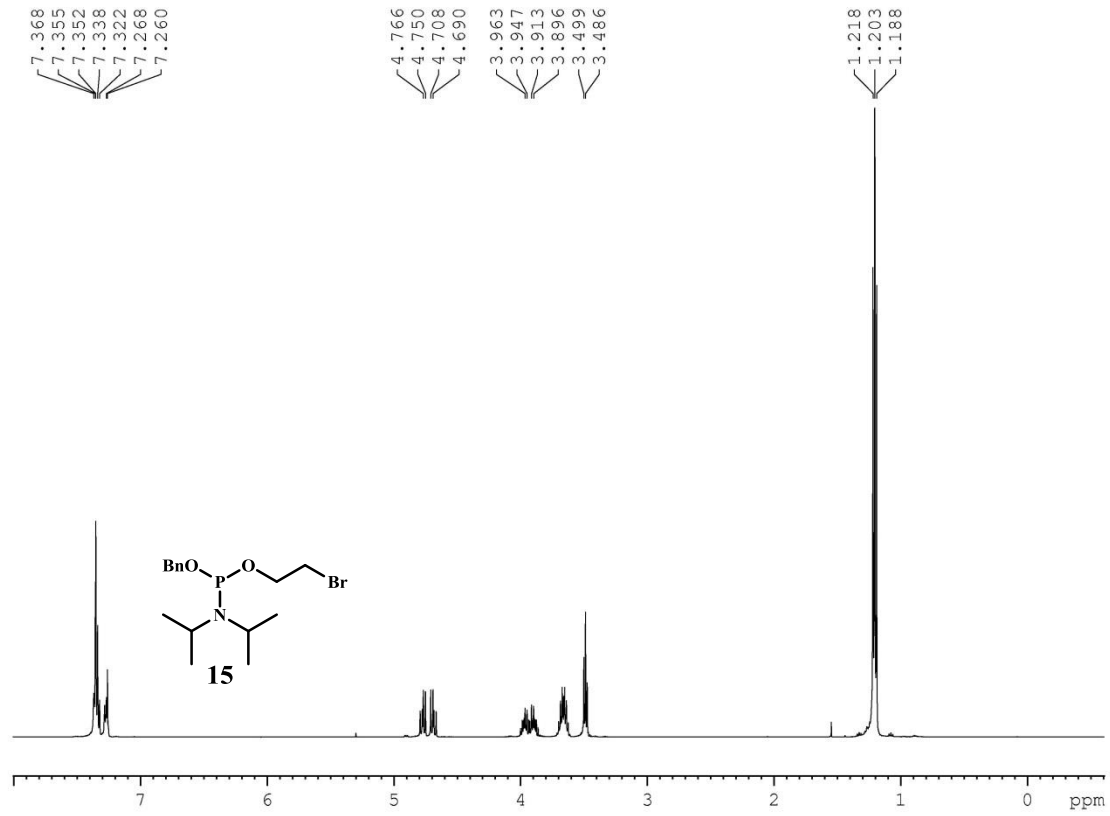
13, ^{31}P NMR

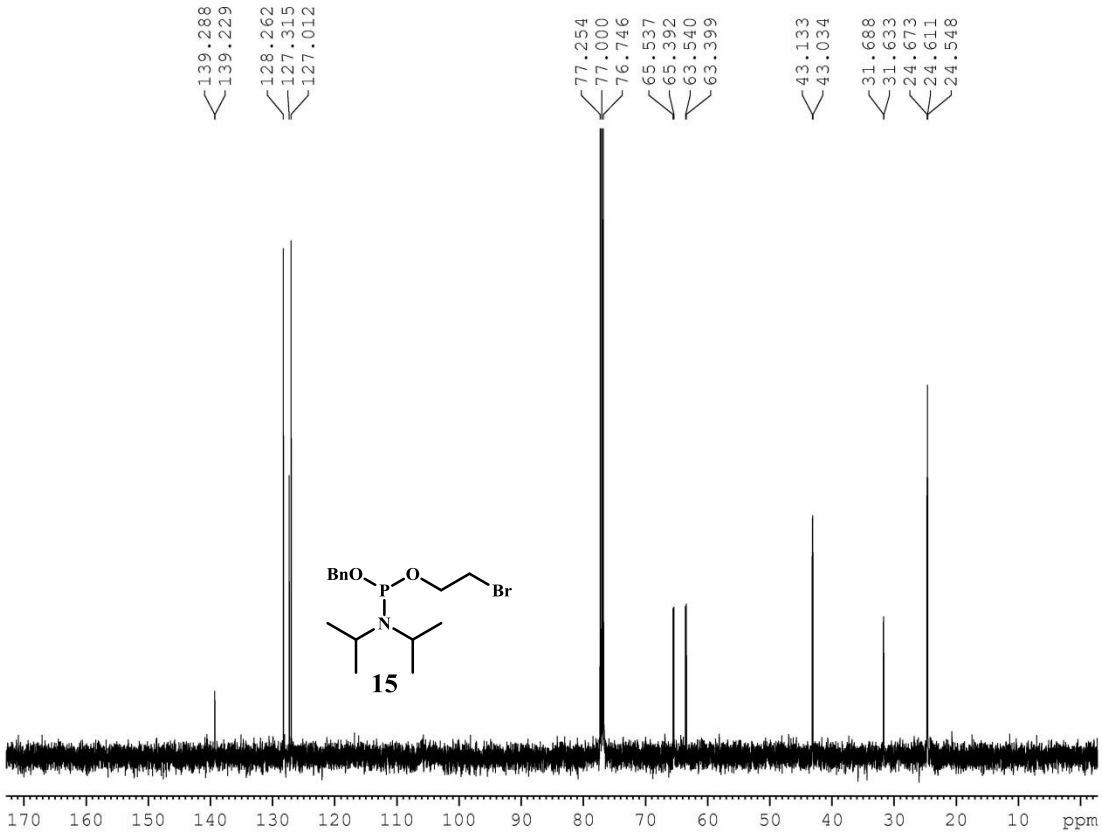

7.260
4.144
4.129
4.088
4.073
3.816
3.802
3.789
3.780
3.775
3.767
3.753
3.739
1.278
1.265
1.226
Cl
P
O
Br
N
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm

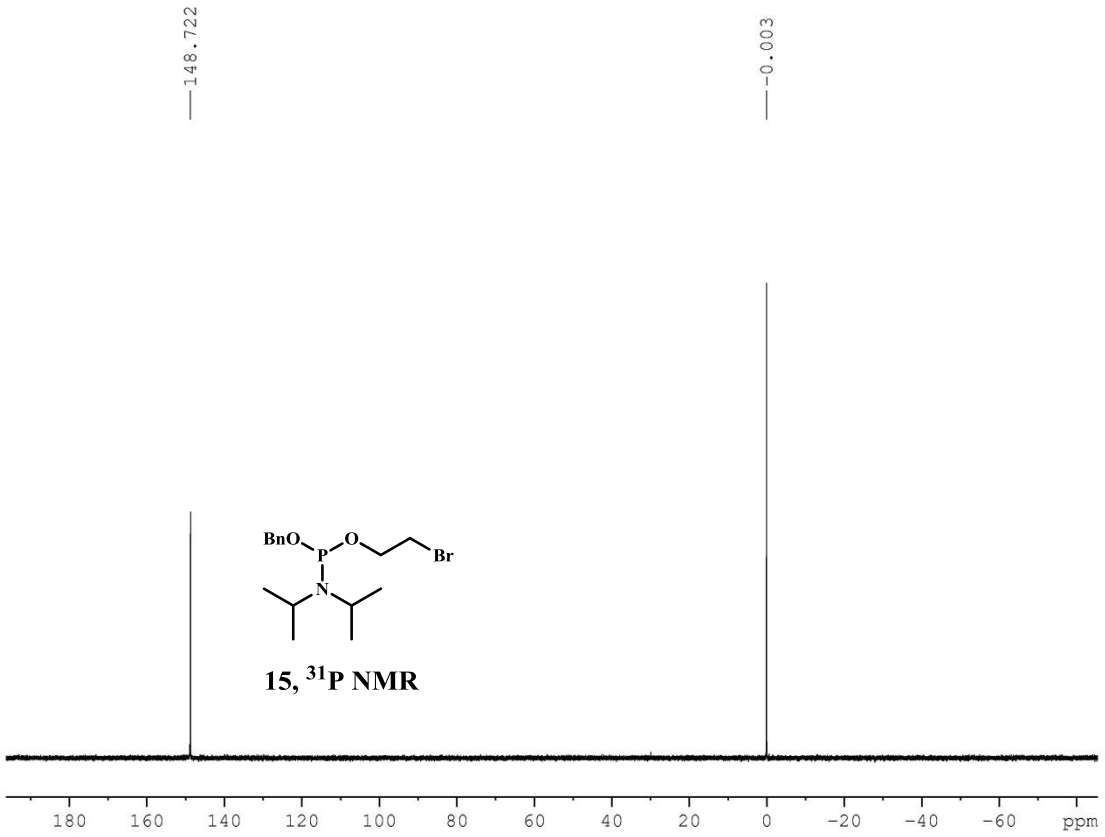
14


77.255
77.001
76.746
65.488
65.349
46.039
45.934
30.553
30.496
23.898
23.244
23.163
Cl
P
O
Br
N
14
85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 ppm
181.335
0.001

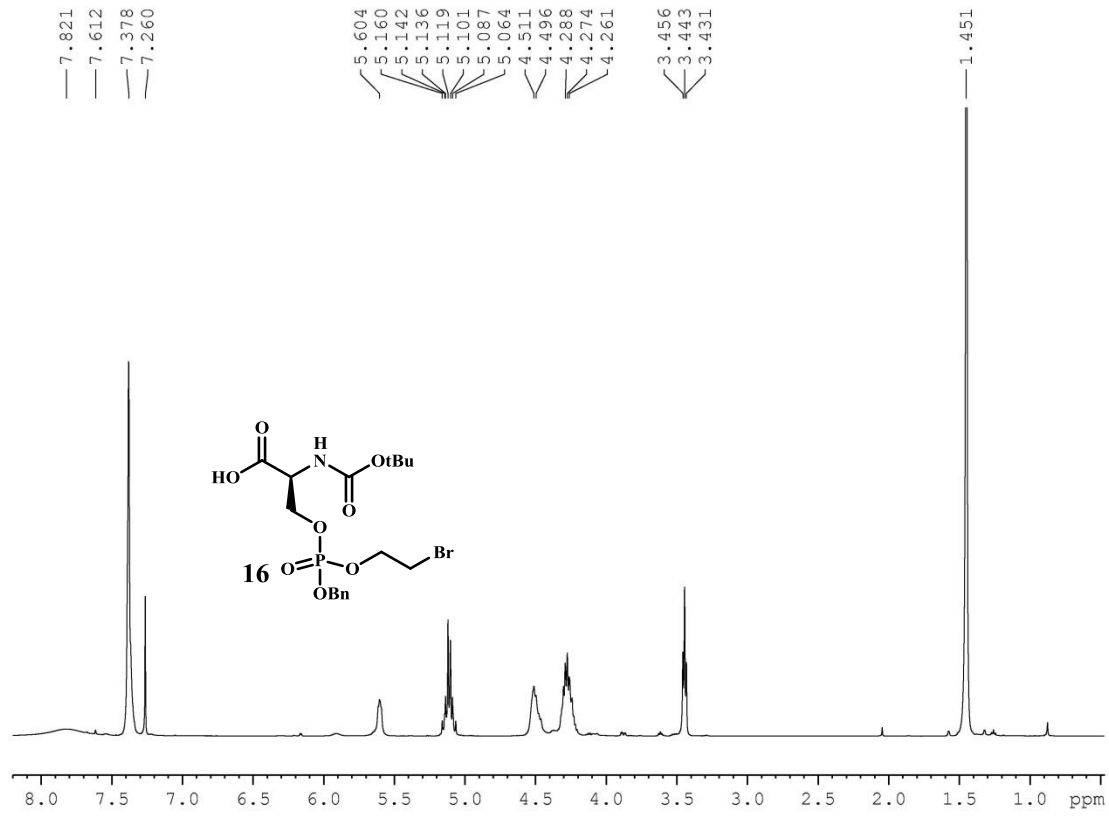

Cl
P
O
Br
N
14, 13P NMR
250 200 150 100 50 0 -50 -100 ppm


7.368
7.355
7.352
7.338
7.322
7.268
7.260
4.766
4.750
4.708
4.690
3.963
3.947
3.913
3.896
3.499
3.486
1.218
1.203
1.188
BnO
P
O
Br
N
15
7
6
5
4
3
2
1
0
ppm

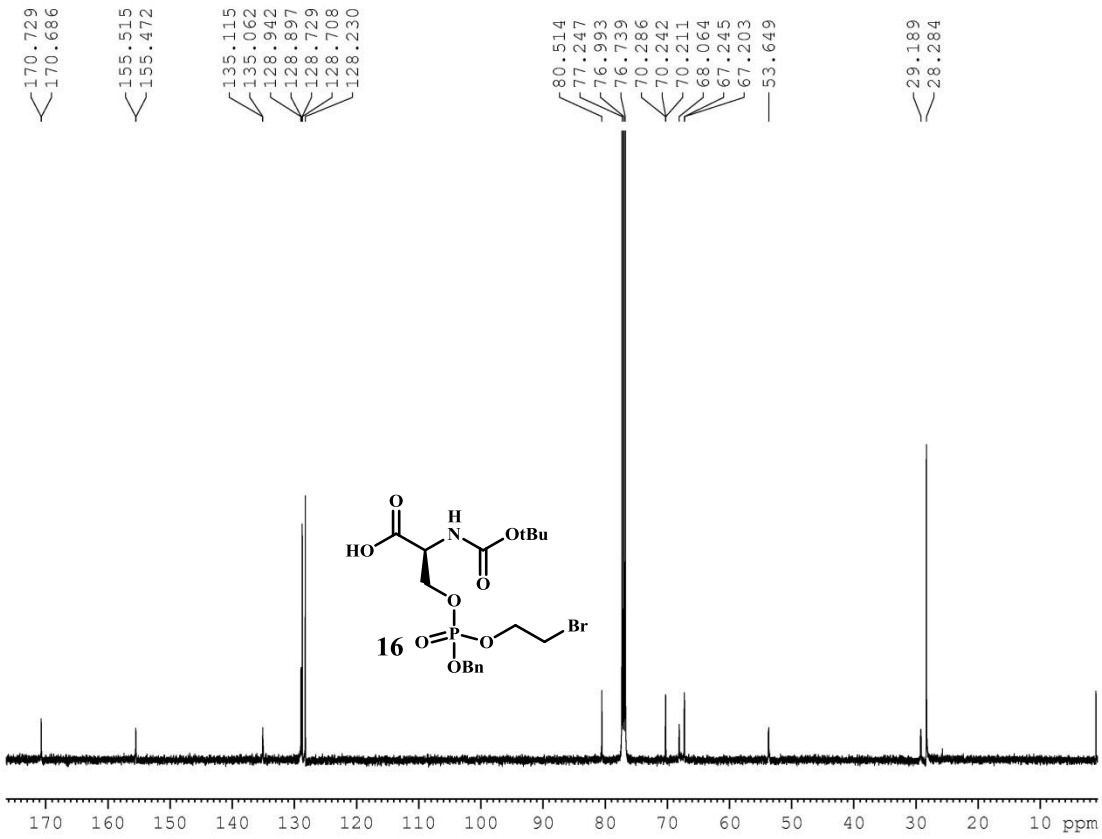

139.288
139.229
128.262
127.315
127.012
77.254
77.000
76.746
65.537
65.392
63.540
63.399
43.133
43.034
31.688
31.633
24.673
24.611
24.548
BnO
P
O
Br
N
15
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

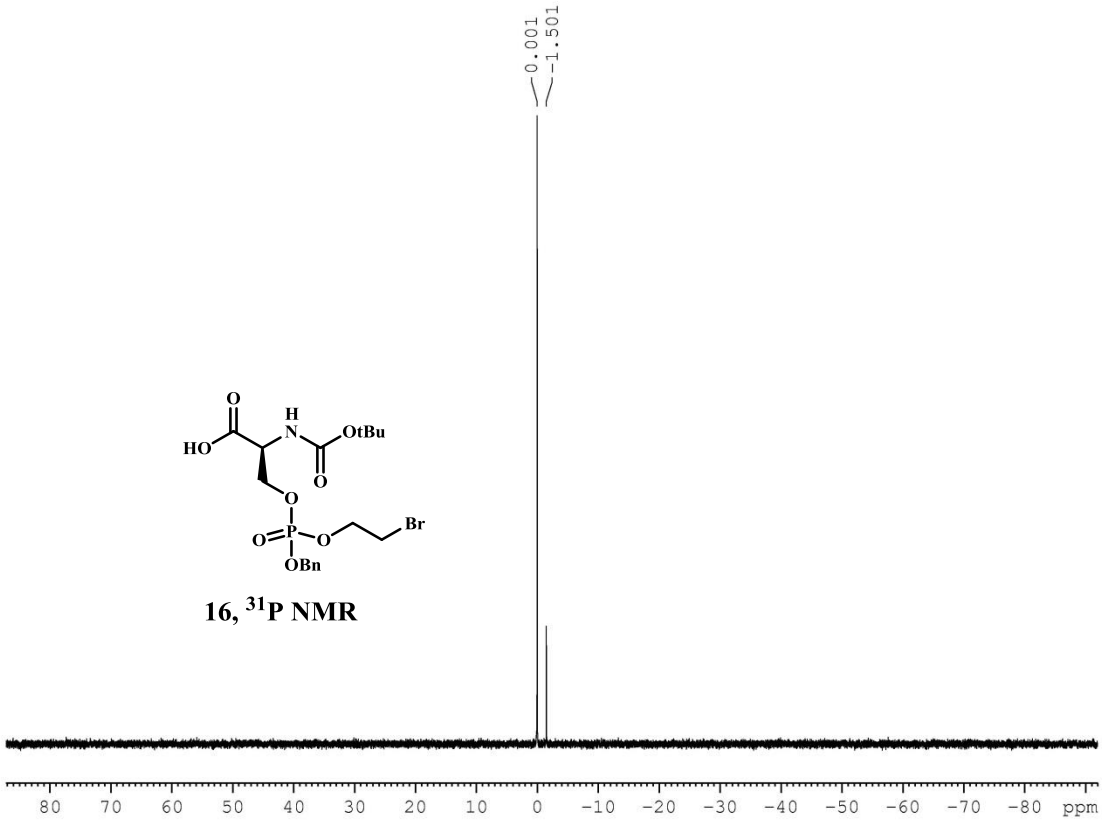

148.722
0.003
BnO
P
O
Br
N
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
ppm

15, ^{31}P NMR

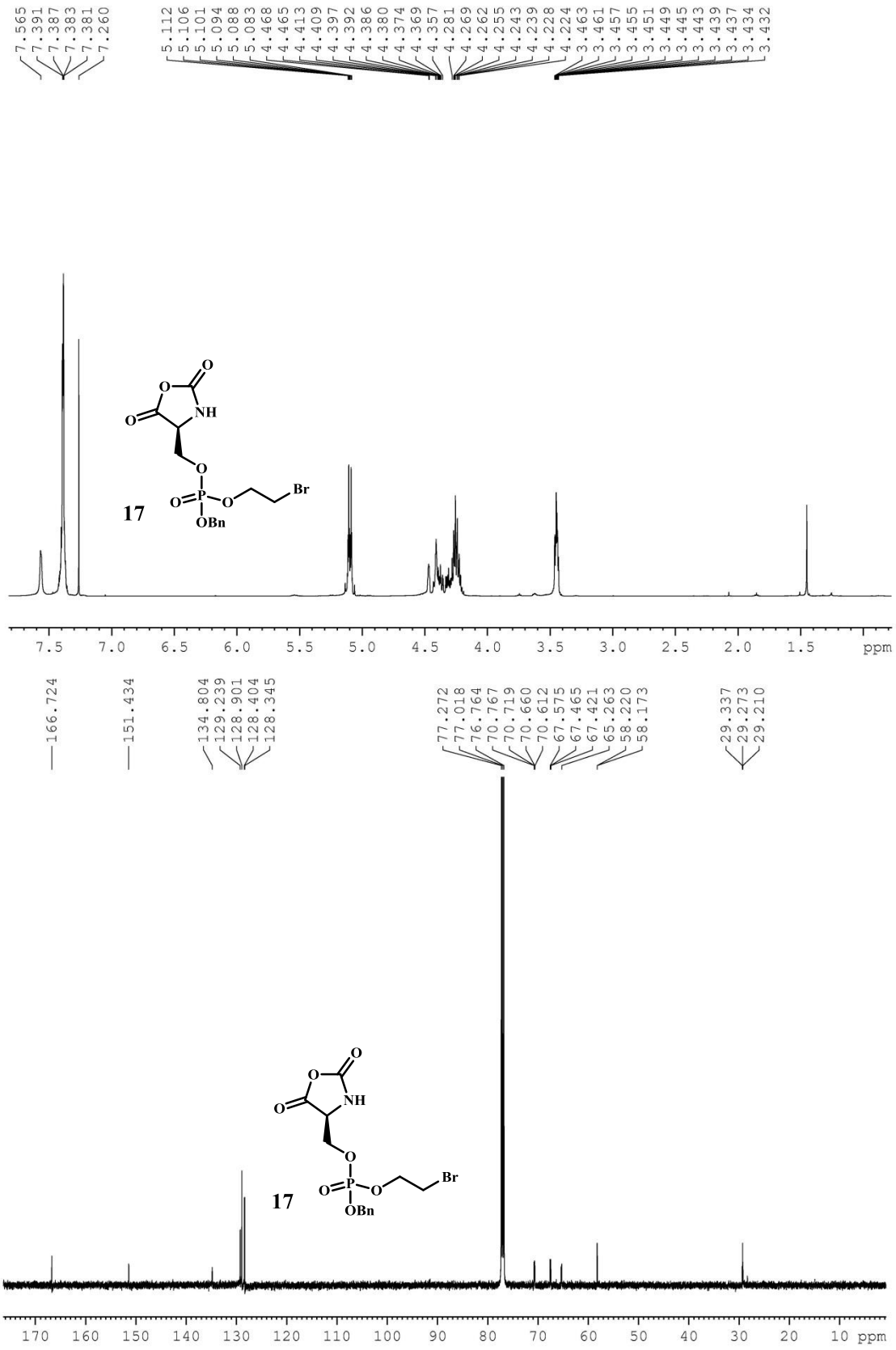

7.821
7.612
7.378
7.260
5.604
5.160
5.142
5.136
5.119
5.101
5.087
5.064
4.511
4.496
4.288
4.274
4.261
3.456
3.443
3.431
1.451
HO
O
H
N
OtBu
O
O
P
O
Br
OBn
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

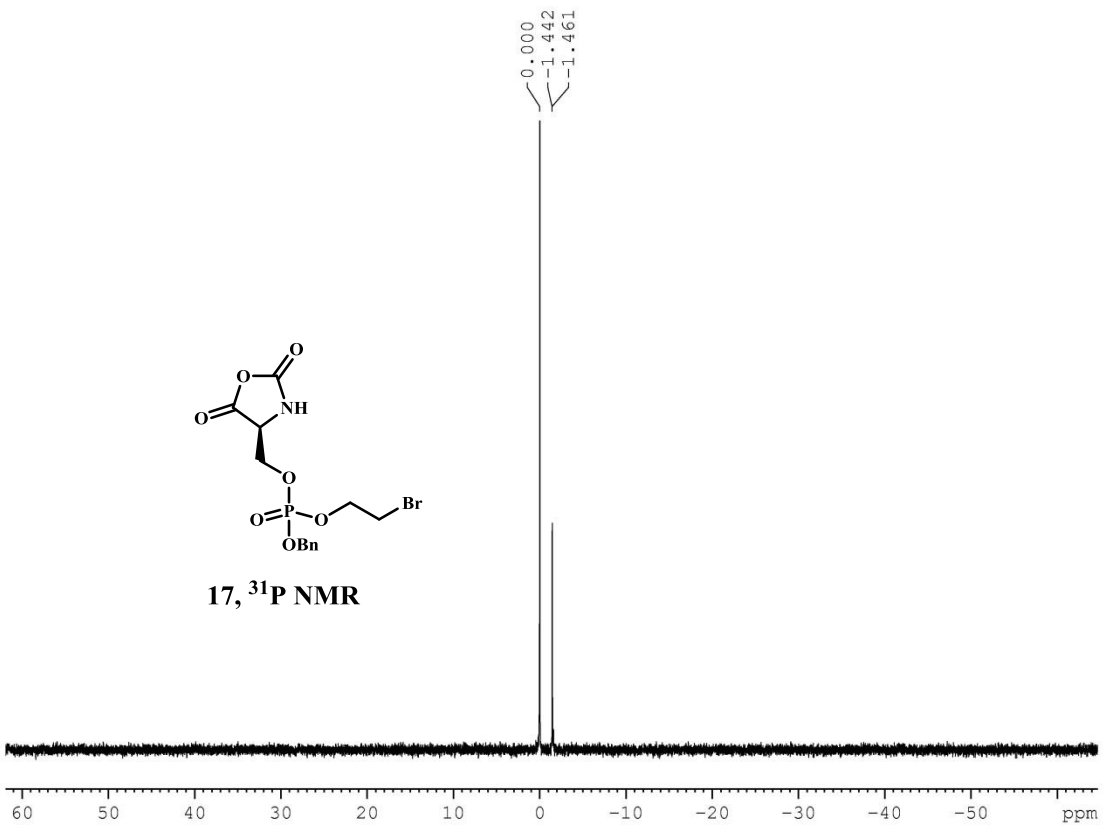
16


170.729
170.686
155.515
155.472
135.115
135.062
128.942
128.897
128.729
128.708
128.230
80.514
77.247
76.993
76.739
70.286
70.242
70.211
68.064
67.245
67.203
53.649
29.189
28.284
16
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

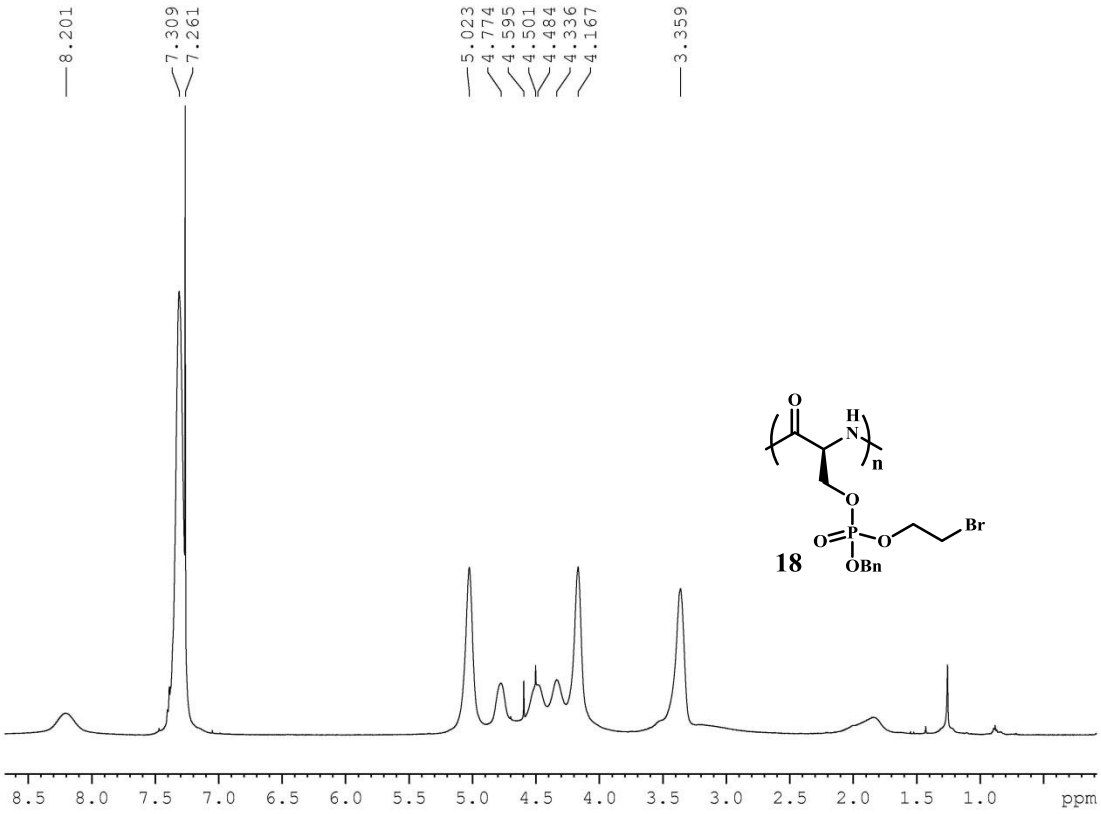

0.001
-1.501
80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 ppm

16, ^{31}P NMR


7.565
7.391
7.387
7.383
7.381
7.260
5.112
5.106
5.101
5.094
5.088
5.083
4.468
4.465
4.413
4.409
4.397
4.392
4.386
4.380
4.374
4.369
4.357
4.281
4.269
4.262
4.255
4.243
4.239
4.228
4.224
3.463
3.461
3.457
3.455
3.451
3.449
3.445
3.443
3.439
3.437
3.434
3.432
17
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
166.724
151.434
134.804
129.239
128.901
128.404
128.345
77.272
77.018
76.764
70.767
70.719
70.660
70.612
67.575
67.465
67.421
65.263
58.220
58.173
29.337
29.273
29.210
17
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10 ppm

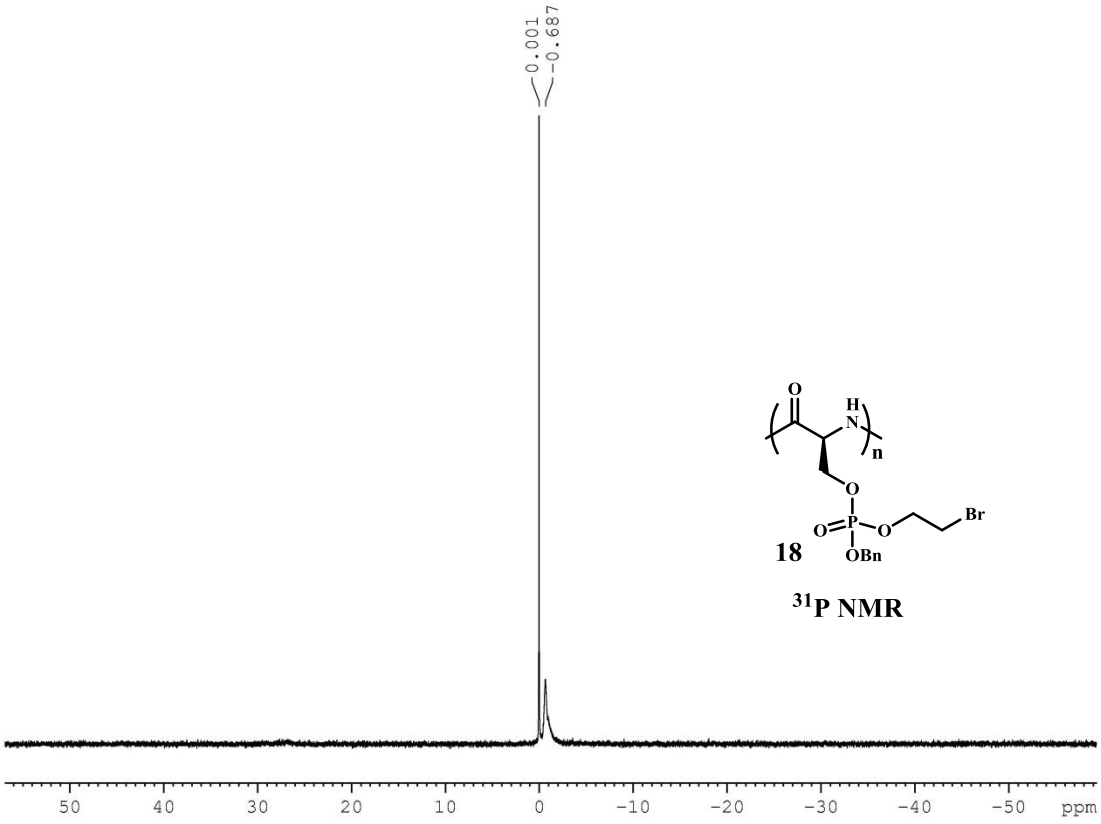

0.000
-1.442
-1.461
O
O
O
NH
O
O
P
O
Br
OBn
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
ppm

17, ^{31}P NMR


8.201
7.309
7.261
5.023
4.774
4.595
4.501
4.484
4.336
4.167
3.359
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm

O
H
N
n
O
O
P
O
Br
O
OBn

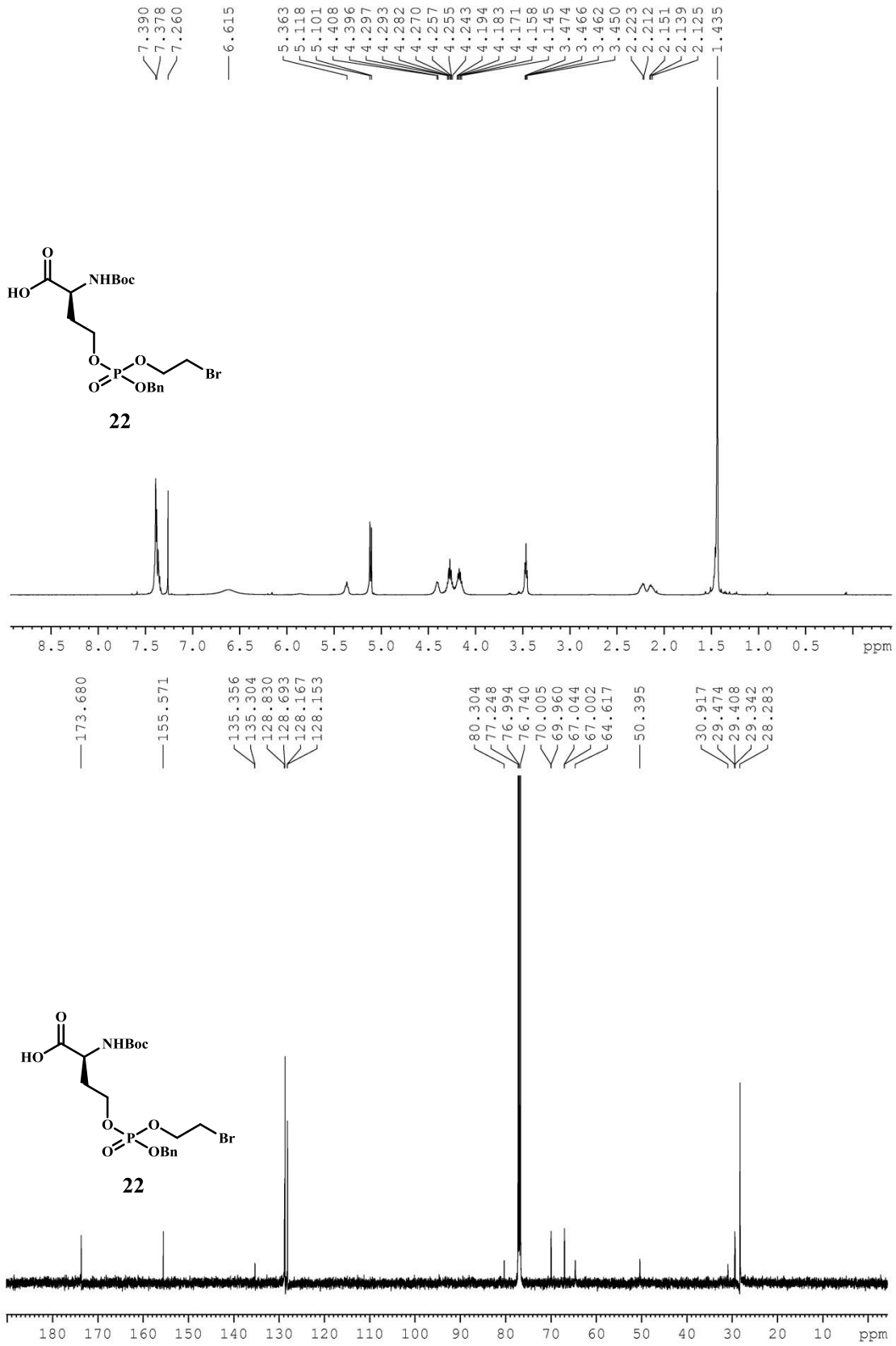
18

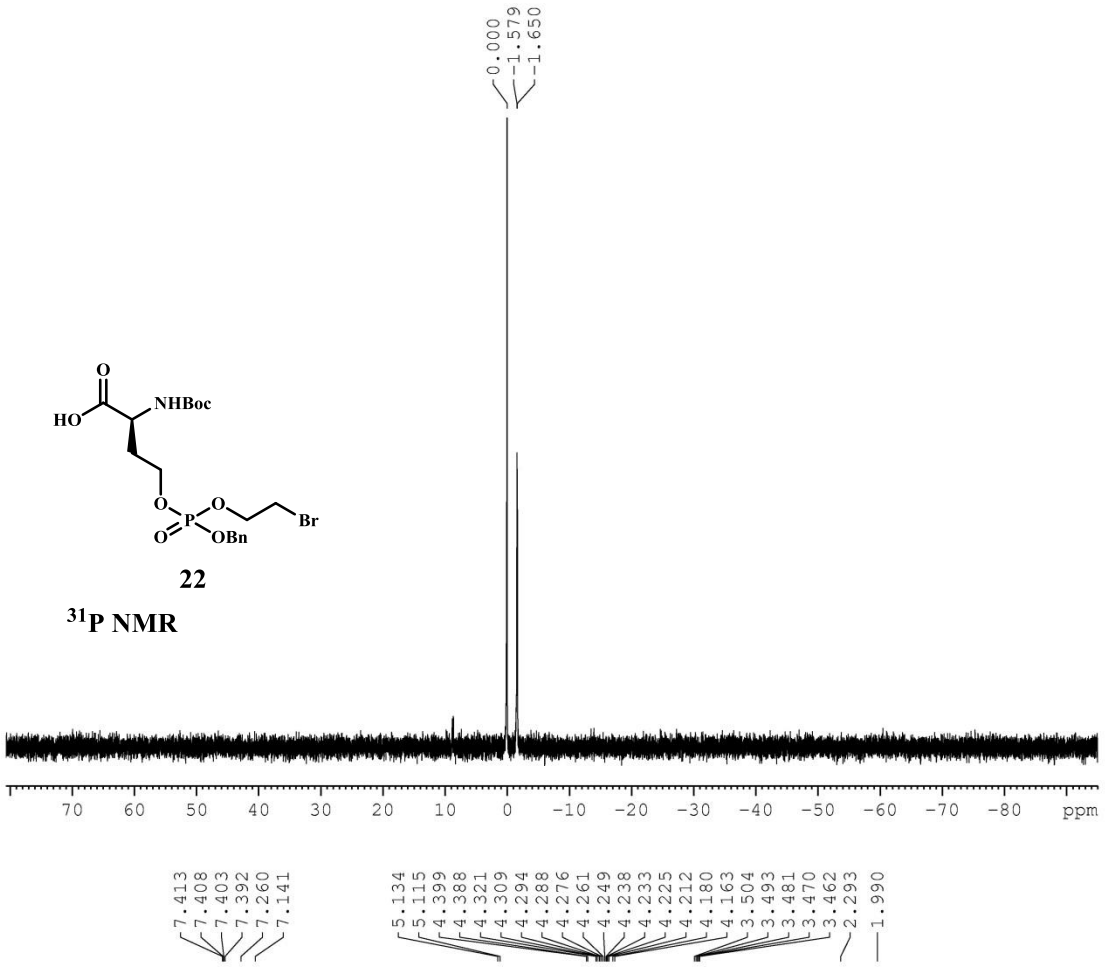

0.001
-0.687
50 40 30 20 10 0 -10 -20 -30 -40 -50 ppm

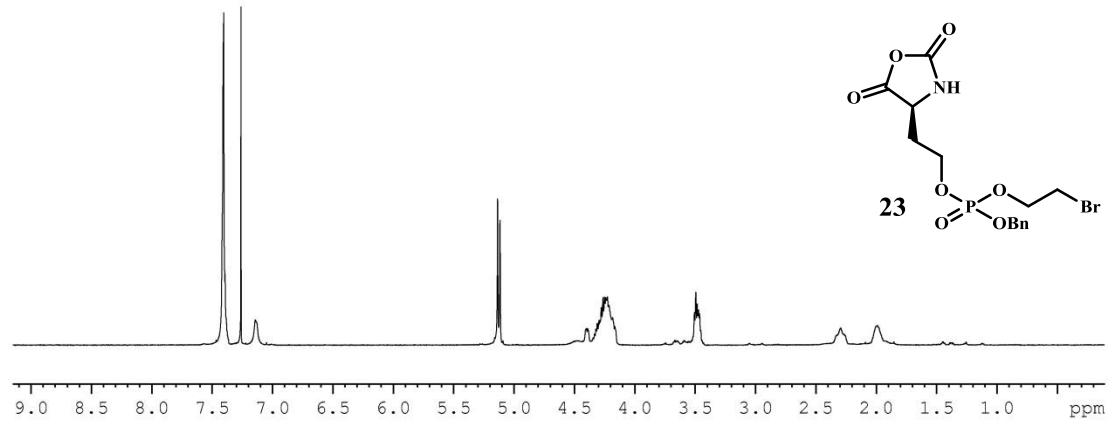
O
H
N
n
O
O
P
O
Br
O
OBn

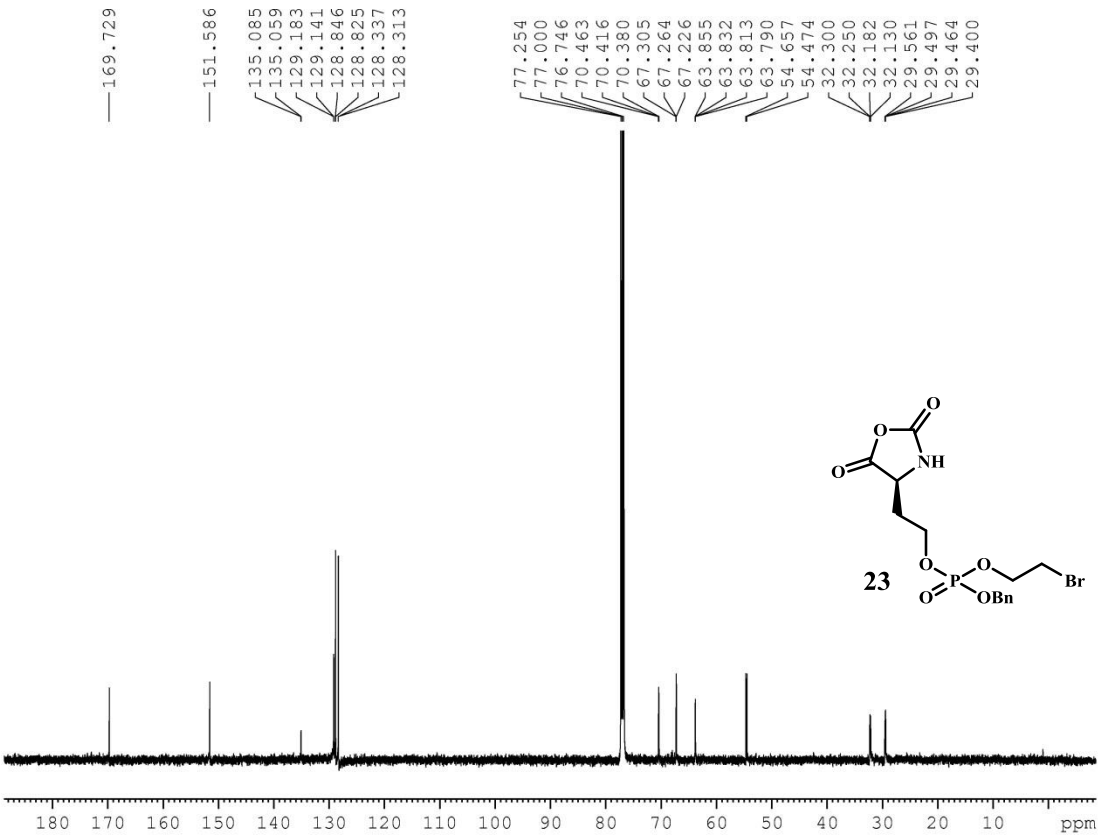
18

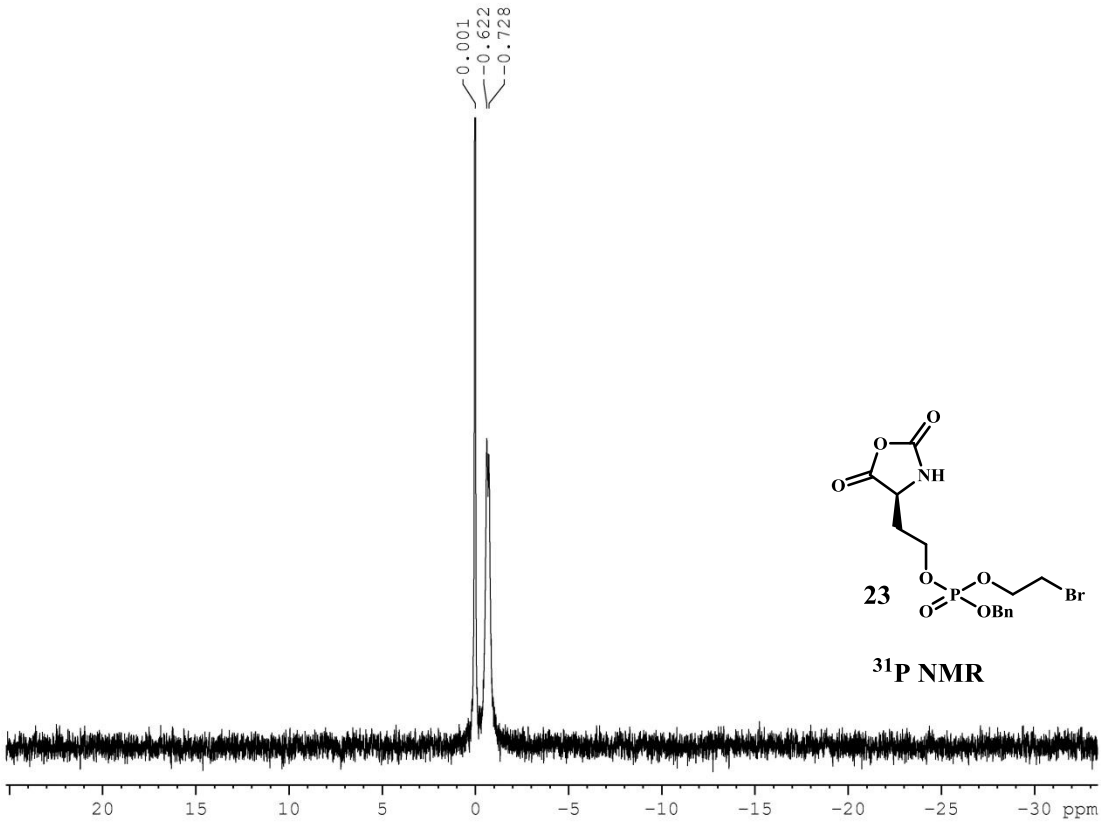
^{31}P NMR

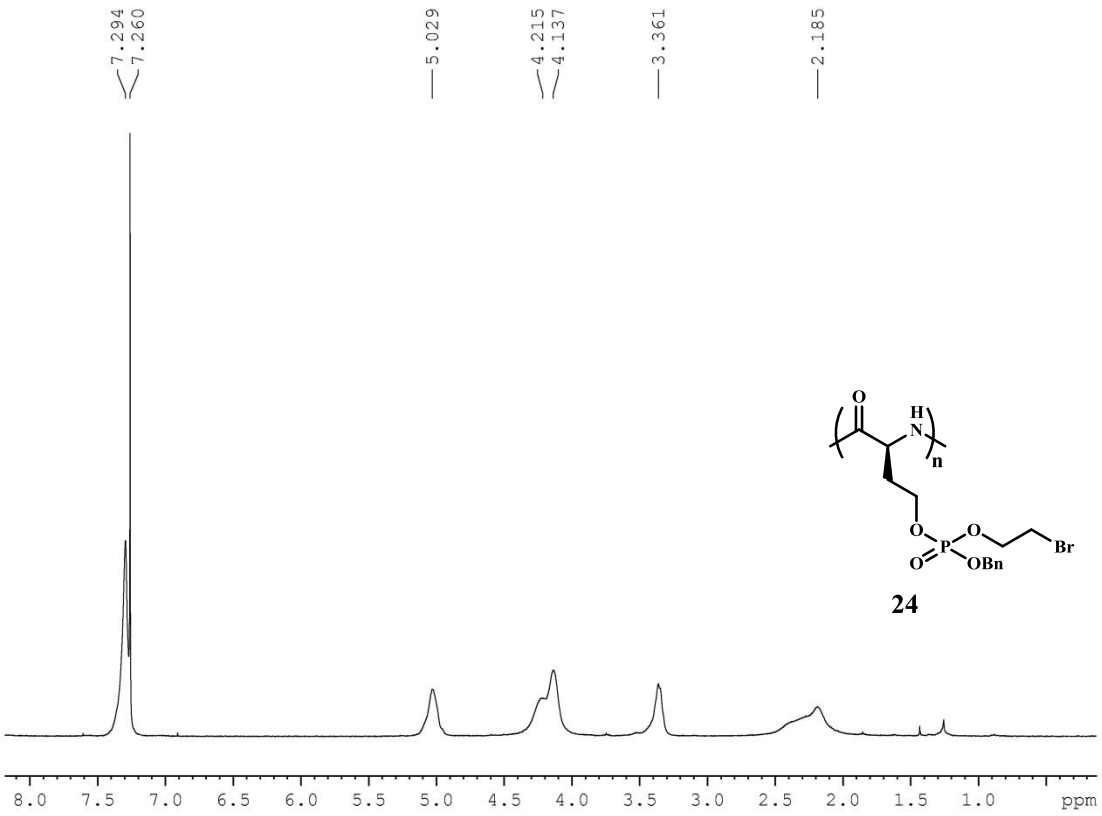

7.390
7.378
7.260
6.615
5.363
5.118
5.101
4.408
4.396
4.297
4.293
4.282
4.270
4.257
4.255
4.243
4.194
4.183
4.171
4.158
4.145
3.474
3.466
3.462
3.450
2.223
2.212
2.151
2.139
2.125
1.435
NHBoc
HO
O
O
P
O
OBn
Br
22
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
173.680
155.571
135.356
135.304
128.830
128.693
128.167
128.153
80.304
77.248
76.994
76.740
70.005
69.960
67.044
67.002
64.617
50.395
30.917
29.474
29.408
29.342
28.283
NHBoc
HO
O
O
P
O
OBn
Br
22
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


0.000
-1.579
-1.650
NHBoc
HO
O
O
P
O
OBn
Br
22
31P NMR
70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 ppm
7.413
7.408
7.403
7.392
7.260
7.141
5.134
5.115
4.399
4.388
4.321
4.309
4.294
4.288
4.276
4.261
4.249
4.238
4.233
4.225
4.212
4.180
4.163
3.504
3.493
3.481
3.470
3.462
2.293
1.990

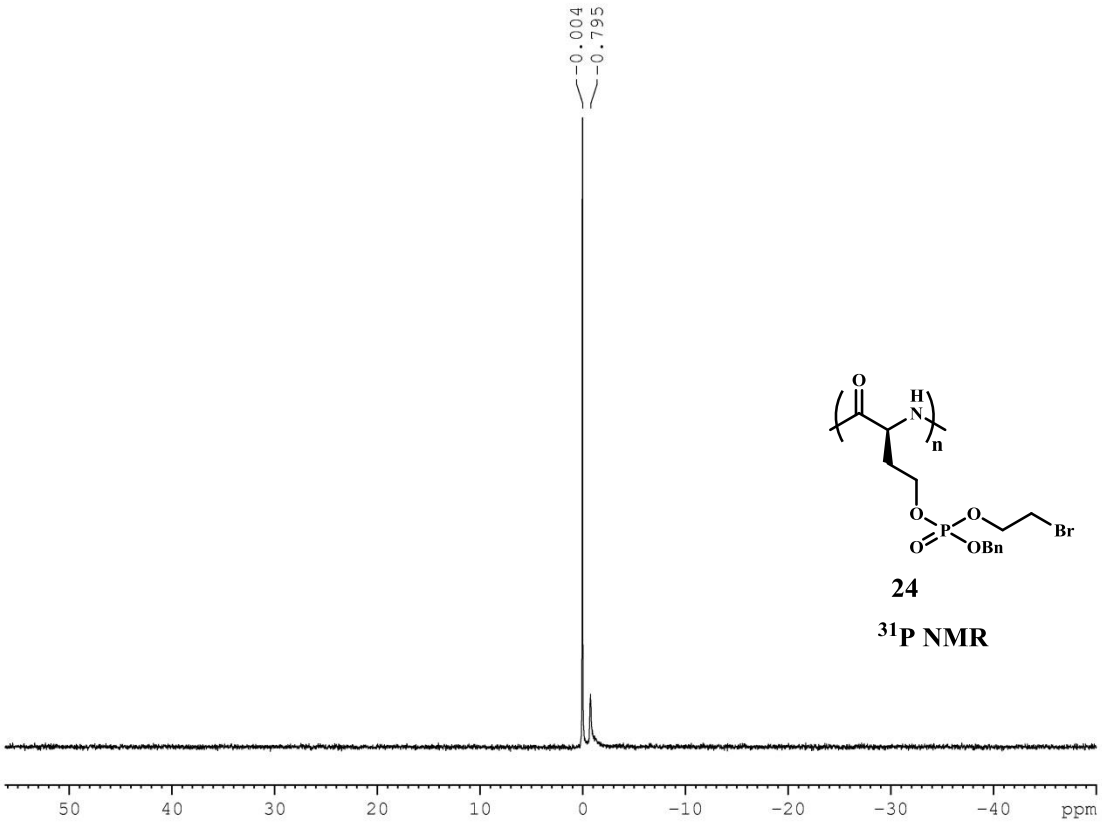

NH
O
O
O
O
P
O
OBn
Br
23
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm


169.729
151.586
135.085
135.059
129.183
129.141
128.846
128.825
128.337
128.313
77.254
77.000
76.746
70.463
70.416
70.380
67.305
67.264
67.226
63.855
63.832
63.813
63.790
54.657
54.474
32.300
32.250
32.182
32.130
29.561
29.497
29.464
29.400
23
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


0.001
-0.622
-0.728
23
31P NMR
20 15 10 5 0 -5 -10 -15 -20 -25 -30 ppm

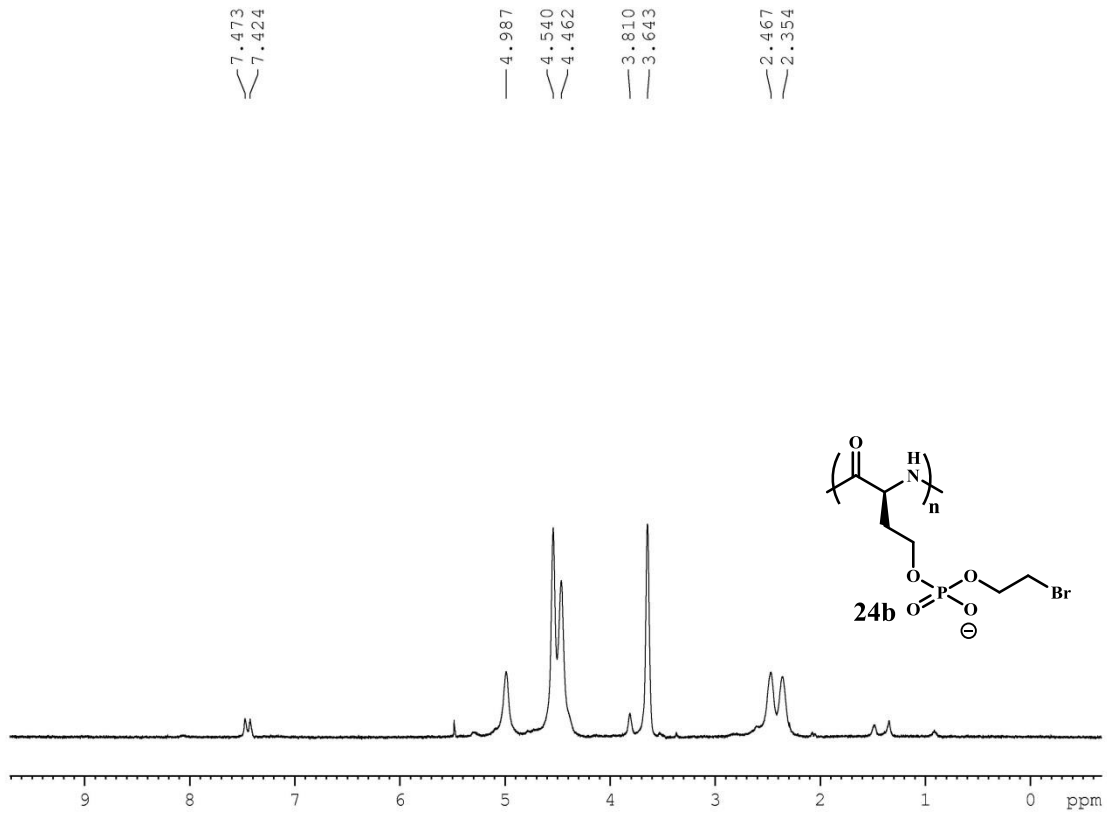

7.294
7.260
5.029
4.215
4.137
3.361
2.185
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

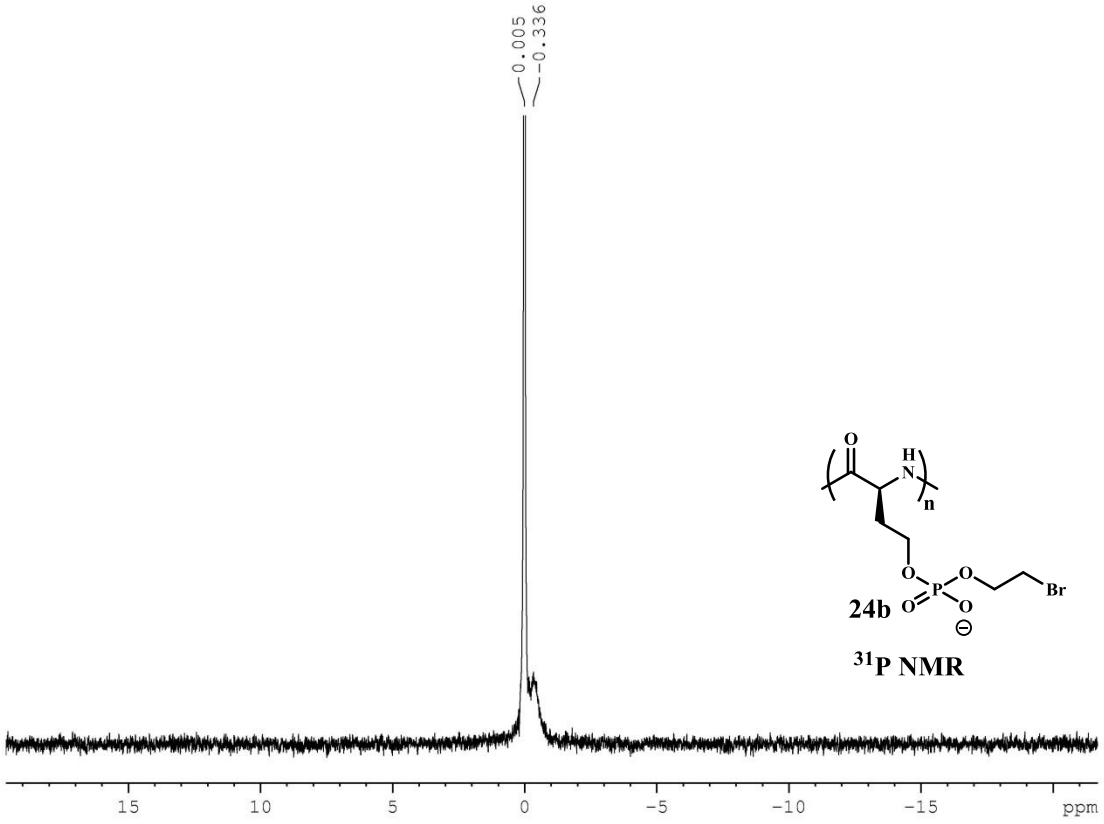
24


-0.004
-0.795
50
40
30
20
10
0
-10
-20
-30
-40
ppm

24

^{31}P NMR


7.473
7.424
4.987
4.540
4.462
3.810
3.643
2.467
2.354
24b
Br
9
8
7
6
5
4
3
2
1
0
ppm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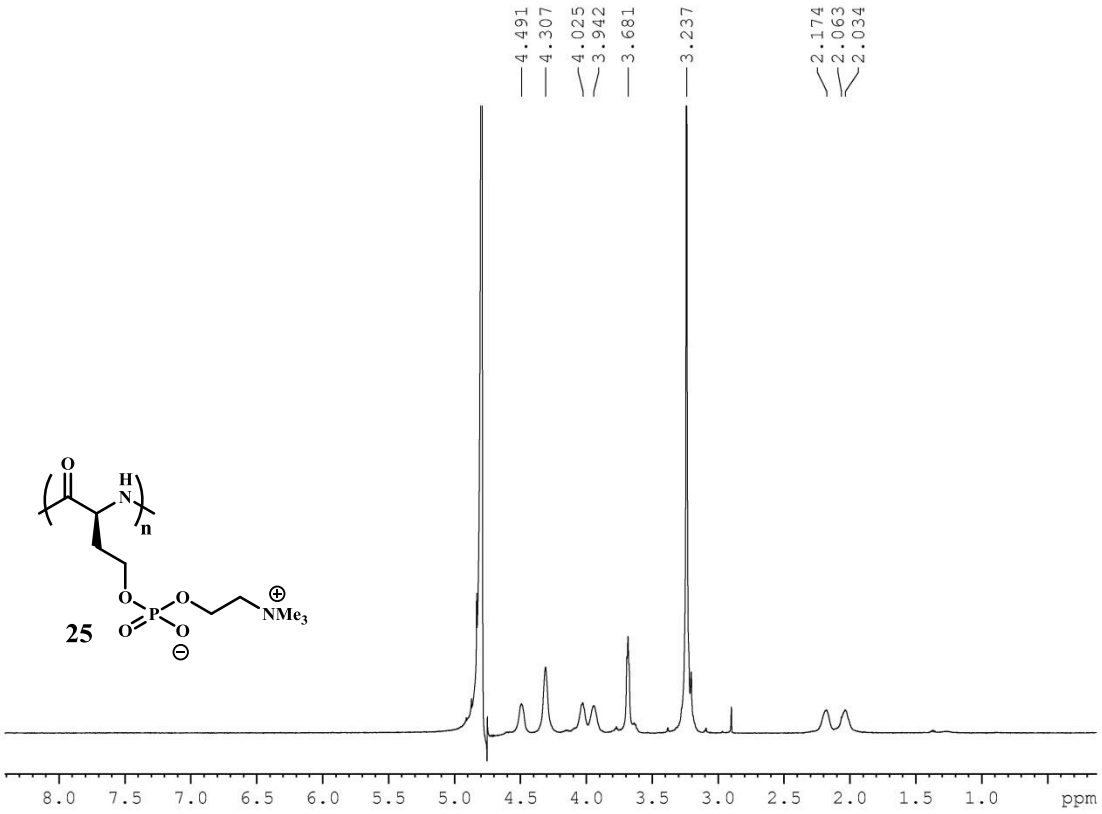
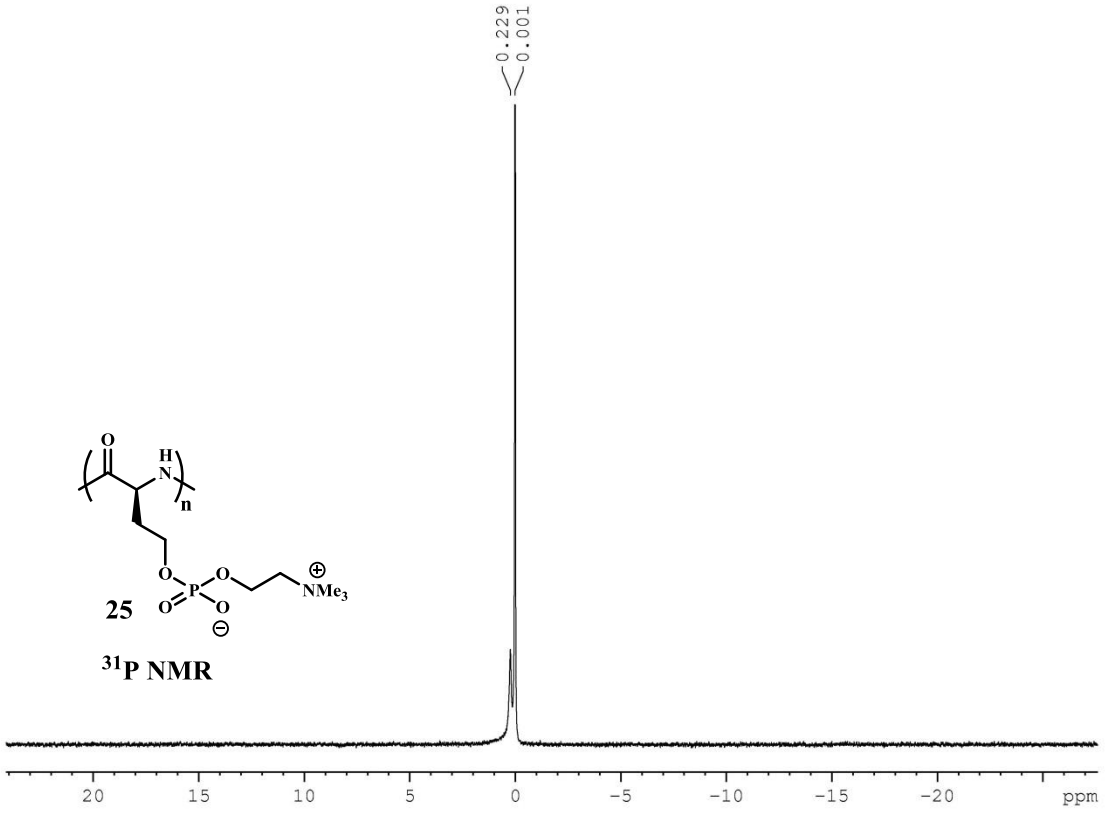
-0.336
24b
Br
31P NMR
15
10
5
0
-5
-10
-15
ppm


4.491
4.307
4.025
3.942
3.681
3.237
2.174
2.063
2.034
25
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


0.229
0.001
25
31P NMR
20
15
10
5
0
-5
-10
-15
-20
ppm

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