

**DESIGN AND SYNTHESIS OF  
AMINOPHOSPHONATES AS PROTEASE INHIBITORS  
AND DEVELOPMENT OF NEW SYNTHETICALLY  
USEFUL METHODOLOGIES**

A THESIS

*Submitted to the*

**UNIVERSITY OF PUNE**

*For the degree of*

**DOCTOR OF PHILOSOPHY**

*in*

**CHEMISTRY**

*by*

**KALPESHKUMAR C. RANA**

*Research Supervisor*

**DR. ASISH K. BHATTACHARYA**

DIVISION OF ORGANIC CHEMISTRY  
NATIONAL CHEMICAL LABORATORY (CSIR)  
PUNE-411 008, INDIA

**MARCH 2011**

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*DEDICATED  
TO  
MY PARENTS  
AND  
TEACHERS*



## राष्ट्रीय रासायनिक प्रयोगशाला

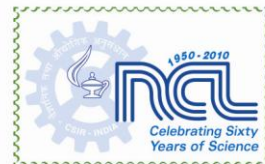
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### CERTIFICATE

This is to certify that the work incorporated in the thesis entitled “*Design and Synthesis of Aminophosphonates as Protease Inhibitors and Development of New Synthetically Useful Methodologies*” which is being submitted to the *University of Pune* for the award of *Doctor of Philosophy in Chemistry* by **Mr. Kalpeshkumar C. Rana** was carried out by him under my supervision at the National Chemical Laboratory, Pune. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

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## **CANDIDATE'S DECLARATION**

I hereby declare that the thesis entitled “*Design and Synthesis of Aminophosphonates as Protease Inhibitors and Development of New Synthetically Useful Methodologies*” submitted by me for the degree of *Doctor of Philosophy in Chemistry* to the *University of Pune* is the record of work carried out by me during the period *August, 2007 to February, 2010* and has not been submitted by me for a degree to any other University or Institution. This work was carried out at Division of Organic Chemistry, National Chemical Laboratory, Pune, India.

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**March 2011**

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*Kalpeshkumar C. Rana*

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## GENERAL REMARKS

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- Independent reference and compound numbering have been employed for each chapter as well as sections of the chapters.
- All the solvents used were purified using the known literature procedures.
- Petroleum ether used in the experiments was of 60-80 °C boiling range.
- Column chromatographic separations were carried out by gradient elution using silica gel (100-200 mesh/230-400 mesh) with light petroleum ether-ethyl acetate mixture, unless otherwise mentioned.
- TLC was performed on E-Merck pre-coated silica gel 60 F254 plates and the spots were rendered visible by exposing to UV light, iodine, charring or staining with ninhydrin, *p*-anisaldehyde solutions in ethanol.
- All the melting points reported are uncorrected and were recorded using Buchi Melting Point apparatus B-540.
- IR spectra were recorded on Shimadzu FTIR instrument, for solid either as nujol mull or in chloroform solution and neat in case of liquid compounds.
- NMR spectra were recorded on Bruker ACF 200 and AV200 (200.13 MHz for <sup>1</sup>H NMR and 50.03 MHz for <sup>13</sup>C NMR), MSL 300 (300.13 MHz for <sup>1</sup>H NMR and 75.03 MHz for <sup>13</sup>C NMR), AV 400 (400.13 MHz for <sup>1</sup>H NMR and 100.03 MHz for <sup>13</sup>C NMR) and DRX 500 (500.13 MHz for <sup>1</sup>H NMR and 125.03 MHz for <sup>13</sup>C NMR) spectrometers. Chemical shifts ( $\delta$ ) reported are referred to internal reference tetramethylsilane (TMS). The following abbreviations were used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = doublet of doublet, dt = doublet of triplet and ddd = doublet of doublet of doublet. Mass spectra were recorded on LC-MS/MS-TOF API QSTAR PULSAR spectrometer, samples introduced by infusion method using Electrosprey Ionization Technique.
- Micro analytical data were obtained using a Carlo-Erba CHNS-O EA 1108 Elemental Analyzer. Elemental analyses observed for all the newly synthesized compounds were within the limits of accuracy ( $\pm 0.4\%$ ).
- Optical rotations were obtained on Bellingham & Stanley ADP-220 Polarimeter. Specific rotations ( $[\alpha]_D$ ) are reported in deg/dm, and the concentration (c) is given in g/100 mL in the specific solvent.

- All the compounds previously known in the literature were characterized by comparison of their  $R_f$  values on TLC, IR and NMR spectra.
- Starting materials were obtained from commercial sources or prepared using known procedures.
- Compounds have been named based on nomenclature provided by Chem Draw Ultra 11.0 software.

## ABBREVIATIONS

---

ACE	Angiotensin-Converting Enzyme
AIDS	Acquired Immunodeficiency Syndrome
ARV	AIDS-Associated Virus
Aq.	Aqueous
AZT	Azidothymidine
BDP	Benzodiazepinylphosphonate
BINOL	1,1'-Bi-2-naphthol
Boc	<i>tert</i> -Butoxycarbonyl
Bn	Benzyl
CAN	Ceric Ammonium Nitrate
Cbz	Benzyloxycarbonyl
ChT	Chymotrypsin
CD4	Cluster of Differentiation 4
DAP	Diallylphosphite
DBP	Dibutylphosphite
DCM	Dichloromethane
DCC	N,N'-Dicyclohexylcarbodiimide
DEAD	Diethyl Azodicarboxylate
DEP	Diethylphosphite
DEPT	Distortionless Enhancement by Polarization Transfer
DHP	Dihydropyran
DIBAL	Diisobutylaluminium Hydride
DIPEA	N,N-Diisopropylethylamine
DMAP	4-(Dimethylamino)pyridine
DMF	Dimethylformamide
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribose Nucleic Acid
ECE	Endothelin converting enzyme
EtOH	Ethanol
gp	Glycoprotein
h	Hour(s)
HEA	Hydroxyethylamine Isostere
HIV	Human Immunodeficiency Virus
HMBC	Heteronuclear Multiple Bond Coherence
HMPA	Hexamethylphosphoric triamide
HNE	Human Neutrophil Elastase
HOBT	Hydroxybenzotriazole
HTLV	Human T Lymphotropic Virus
Hz	Hertz
IR	Infra Red
LAH	Lithium Aluminum Hydride
LAV	Lymphadenopathy-Associated Virus
<i>m</i> CPBA	<i>m</i> -Chloroperoxybenzoic acid
MCR	Multicomponent Reaction
MeCN	Acetonitrile
MeOH	Methanol
min.	Minute(s)

mL	Millilitre(s)
μM	Micromolar
mmol	Millimole(s)
MMP	Matrix Metalloproteinase
Mp	Melting Point
MS	Mass Spectrum
MS 4Å	Molecular Sieves (4Å)
MsCl	Mesyl Chloride
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MWI	Microwave Irradiation
NMMO	N-Methylmorpholine N-oxide
NMR	Nuclear Magnetic Resonance
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
ORTEP	Orthogonal Thermal Ellipsoid Plots
PMP	<i>p</i> -Methoxyphenyl
PPE	Procine Pancreatic Elastase
<i>p</i> TSA	<i>p</i> -Toluenesulfonic acid
<i>p</i> TsCl	<i>p</i> -Toluenesulfonyl chloride
Py	Pyridine
RNA	Ribonucleic Acid
rt	Room Temperature
RT	Reverse Transcription
TBAB	Tetrabutylammonium bromide
TBAF	Tetra- <i>n</i> -butylammonium fluoride
TCCA	Trichloroisocyanuric Acid
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMSCl	Trimethylchlorosilane
TMSCN	Trimethylsilylcyanide
UA	Ursolic Acid
UNAIDS	Joint United Nations Programme on HIV/AIDS
WHO	World Health Organization

## Abstract

---

The thesis entitled “Design and Synthesis of Aminophosphonates as Protease Inhibitors and Development of New Synthetically Useful Methodologies” consists of four chapters.

### **Chapter 1: General Introduction of Protease Inhibitors and Literature Review of Aminophosphonates**

This chapter reviews reported literature on the protease inhibitors and aminophosphonates. This chapter is divided into two sections.

#### **Section A: General Introduction of Protease Inhibitors**

Proteases are enzymes that catalyze the cleavage of proteins by hydrolysis of peptide bonds. Protease inhibitors are the molecules which inactivate or slow down the enzyme activity. This chapter describes some of the better studied inhibitors of aspartic, serine, cysteine and metallo proteases with respect to active site of the enzyme, chemical structure of the inhibitors and biological significance of the inhibitors.

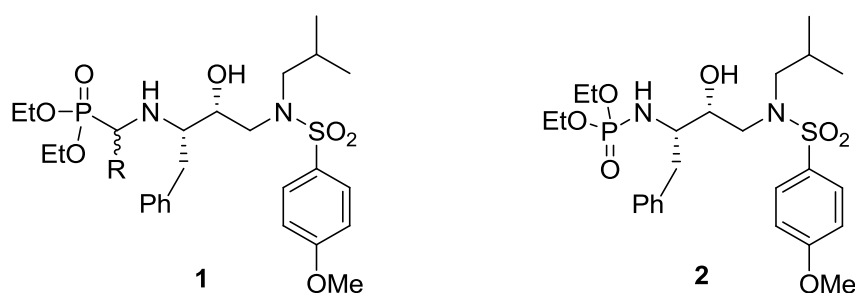
#### **Section B: Literature Review of Aminophosphonates**

Aminophosphonates are esters of the aminophosphonic acids which are structural analogues of amino acids and are classified as  $\alpha$ ,  $\beta$ , and  $\gamma$ -aminophosphonates. Being the structural analogues of amino acids, aminophosphonates act as their antagonist and compete with their carboxylic counterparts for the active site of enzyme or other cell receptor. Therefore, they exert various biological activities which include enzyme inhibitors, neurophysical effects and plant growth inhibitors etc. This section deals with the literature review of aminophosphonates which is further divided into subclasses *e.g.*  $\alpha$ -aminophosphonate,  $\beta$ -aminophosphonate and  $\gamma$ -aminophosphonates. Each class of aminophosphonates is discussed with respect to their syntheses and biological activities.

## Chapter 2: Synthesis of Aminophosphonate Derivatives of Hydroxyethylamine (HEA) Isostere and Their Anti-HIV Activity

Acquired immune deficiency syndrome (AIDS) is a disease of the human immune system caused by the human immunodeficiency virus (HIV). In 2008, it was estimated that 2.31 million people lived with the disease in India alone. Generally the HIV therapy consisted of nucleoside reverse transcriptase inhibitor and HIV protease inhibitors. A series of HIV-1 protease inhibitors gained approval and at present, many drugs are being used clinically such as amprenavir, saquinavir, nelfinavir, indinavir and ritonavir.

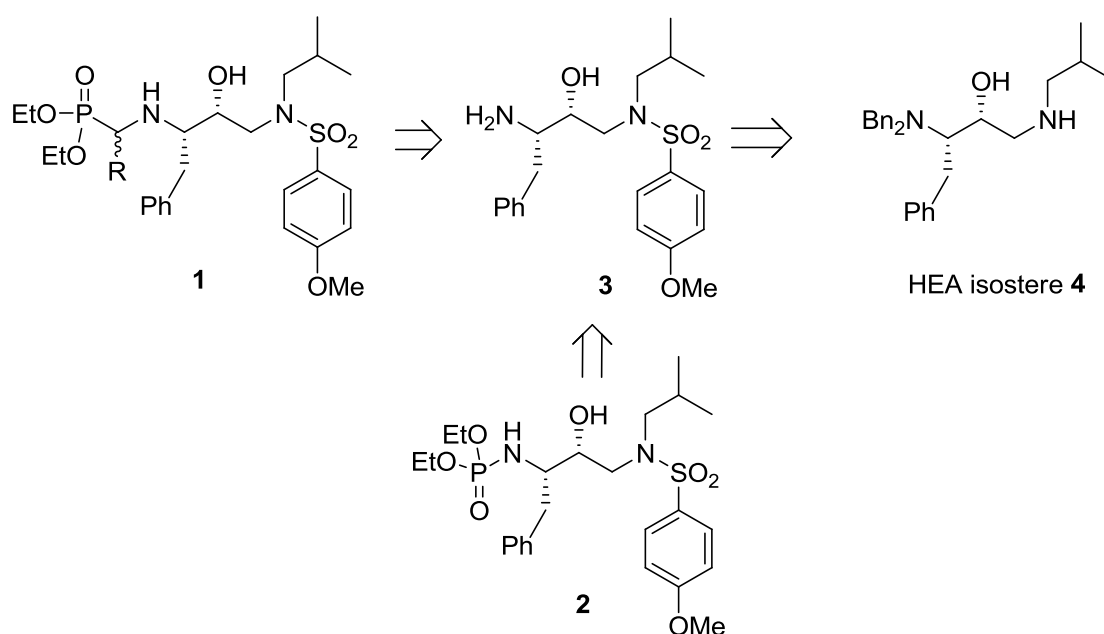
HIV protease possesses a  $C_2$ -symmetric homodimeric structure which selectively cleaves the Phe-Pro (Tyr-Pro) moiety of the virus protein, the rational design of inhibitors proved possible based on substrate models which mimic the transition state of the peptide hydrolysis. Out of these substrate models hydroxyethylamine (HEA) is an attractive motif due to its low molecular weight and it is the central core of several anti-HIV drugs *e.g.* amprenavir. We opined that replacement of  $P_2$  pocket group of the inhibitor with aminophosphonate could result in the more potent inhibitors as phosphonates are known to be stable under physiological conditions, do not react with enzymes like cholinesterase thus decreasing their toxicity, show good cell permeability and increases cellular accumulation and retention of drug compounds, thus improving their therapeutic and diagnostic value. Keeping this in mind, we designed aminophosphonate derivatives of HEA isostere **1** and phosphoramidate derivatives **2** (Figure 1) expecting that aminophosphonate will find its place in  $P_2$  pocket of the enzyme and therefore, derivatives **1** and **2** could serve as potent anti-HIV agents.



**Figure 1.** Designed aminophosphonates and phosphoramidate derivatives of HEA isostere.

Aminophosphonate derivatives of HEA isostere **1** and phosphoramidate derivatives **2** could be synthesized from corresponding amine **3** which in turn could be assessed from HEA isostere **4** as depicted in Scheme 1.

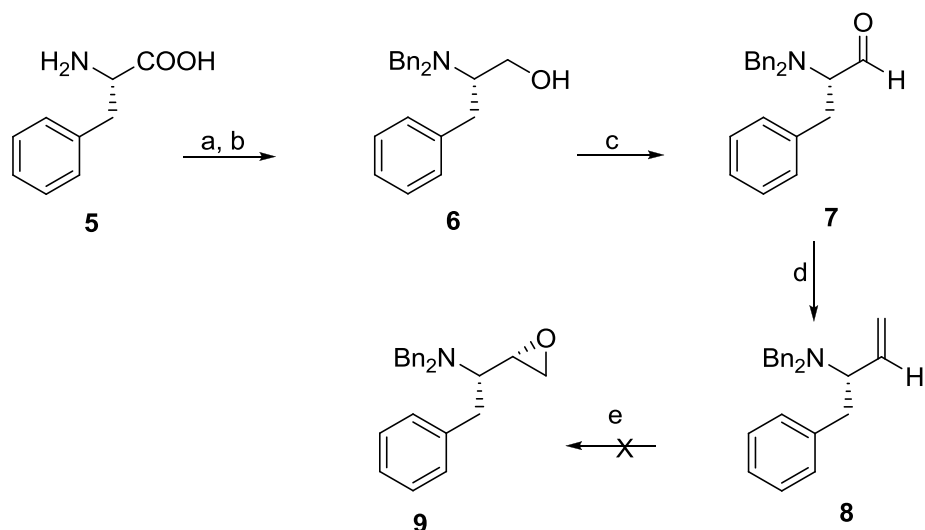
Several methods have been reported for the synthesis of HEA isostere **4** in the literature however, these methods suffer from one or more drawbacks *viz.* low overall yields, lengthy steps and use of hazardous reagents such as lithium metal. Therefore, we carried out the present work which incorporates (i) a new and efficient method for the synthesis of hydroxyethylamine isostere and (ii) synthesis of  $\alpha$ -aminophosphonate and phosphoramidate derivatives of HEA isostere.



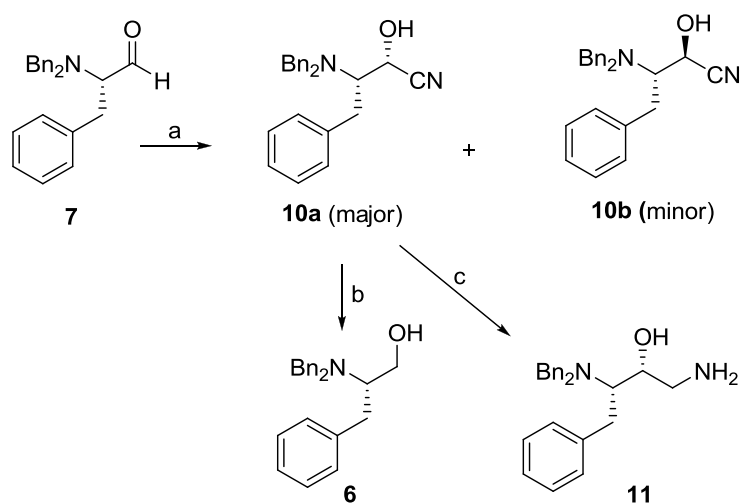
**Scheme 1.** Retrosynthesis of aminophosphonates **1** and phosphoramidate **2** derivatives of HEA isostere.

Three different approaches *i e* epoxidation approach (Scheme 2), reduction of cyanohydrins approach (Scheme 3) and one pot reduction-transimination-reduction approach (Scheme 4) were tried and we were able to synthesized HEA isostere **4** in good yield in third approach.

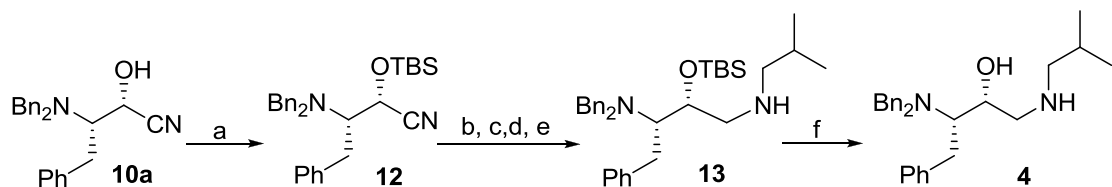




**Scheme 2.** Reagents and conditions: (a) NaOH,  $K_2CO_3$ ,  $H_2O$ , BnBr, reflux, 3 h; (b) LAH,  $Et_2O$ ,  $0^\circ C$ , overnight, 60% after two step; (c)  $(COCl)_2$ , DMSO, DCM,  $-78-0^\circ C$ , TEA, 1 h, 98%; (d) Tebbe reagent, THF,  $0^\circ C$ , 30 min, 63%; (e) *m*CPBA,  $0^\circ C$ , DCM.

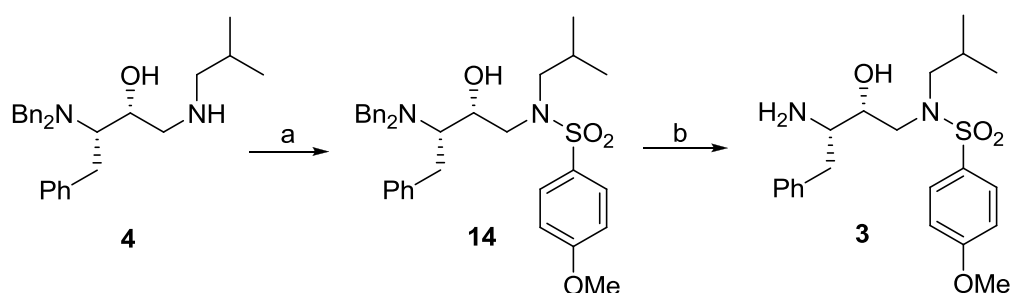


**Scheme 3.** Reagents and conditions: (a) TMS-CN,  $BF_3 \cdot Et_2O$ , DCM,  $-20^\circ C$ , 2 h, 80%; (b) LAH, THF,  $0^\circ C$ , 1 h, 60%; (c)  $NiCl_2 \cdot 6H_2O$ ,  $NaBH_4$ , MeOH,  $0^\circ C$ , 1 h, 50%.

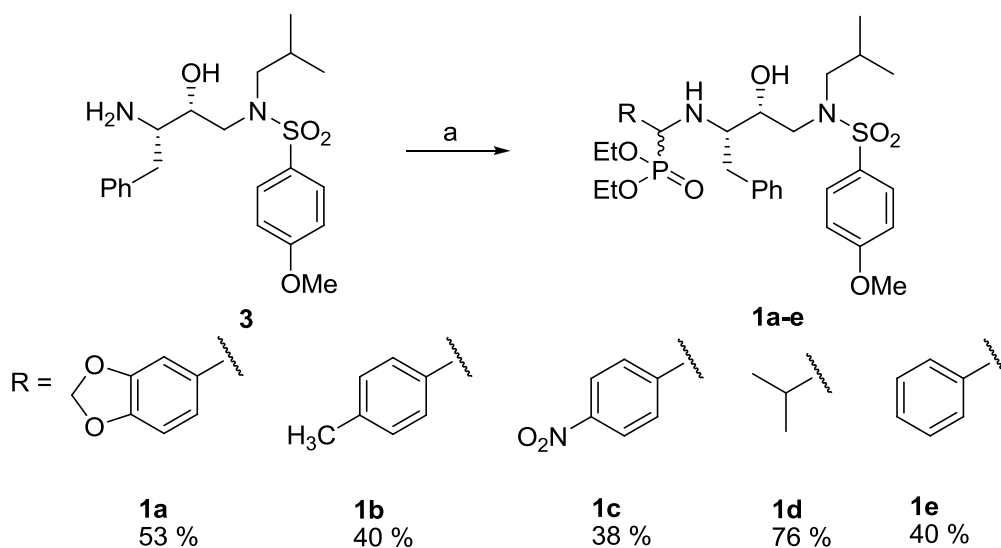


**Scheme 4.** Reagents and conditions: (a) TBS-Cl, Imidazole, DCM, rt, overnight, 93%. (b) DIBAL-H,  $Et_2O$ ,  $-78^\circ C$ , 3 h; (c)  $NH_4Br$  in MeOH; (d) Isobutyl amine, 3 h; (e)  $NaBH_4$ , 75% in four steps; (f) TBAF, THF, 85%.

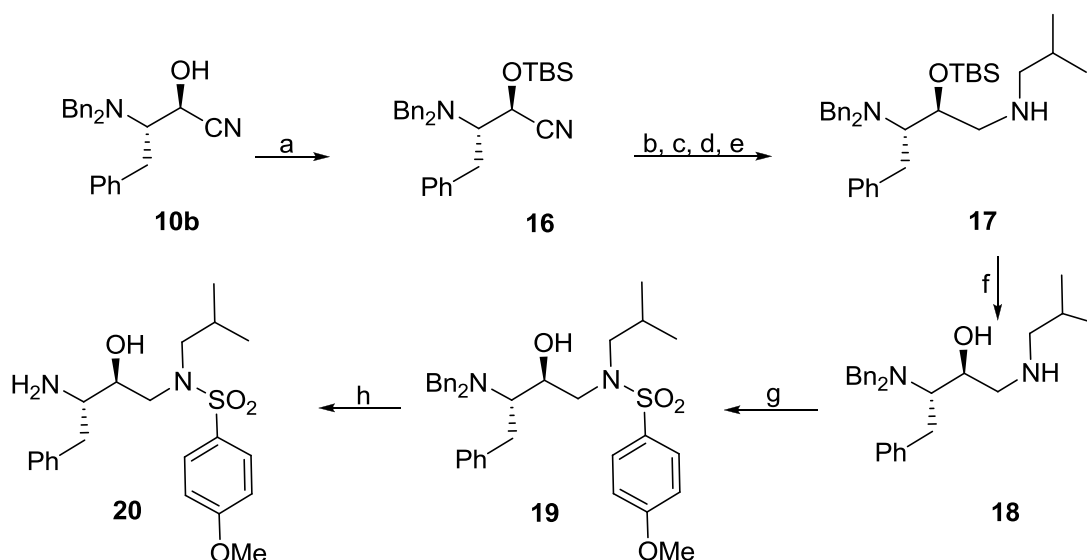
After synthesizing the HEA isostere **4** the designed derivatives **1** & **2** were synthesized as shown in Scheme 5 and Scheme 6. The isostere **4** was subjected to *N*-sulfonylation followed by hydrogenolysis furnished amine **3**. The amine **3** was subjected to Kabachnik-field reaction to furnish corresponding  $\alpha$ -aminophosphonate derivatives **1a-e**. Similar derivatives **21a-e** with  $\beta$ -OH were also synthesized from the minor diastereomer **10b** (Scheme 7 and Scheme 8). Moreover, phosphoramidate derivatives **2a-b** of the amine **3** were also synthesized using Atherton-Todd reaction (Scheme 9).



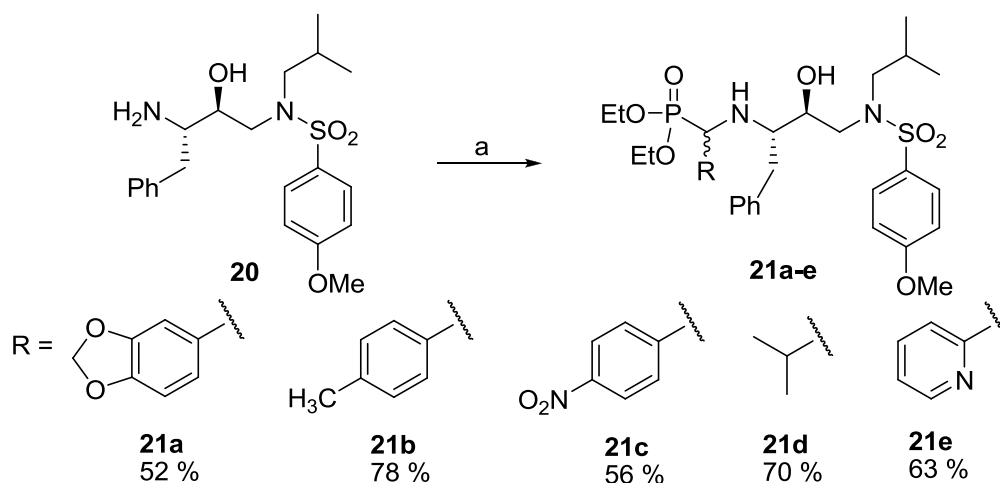
**Scheme 5.** Reagents and conditions: (a) 4-Methoxysulfonylchloride, DCM, aq. Na<sub>2</sub>CO<sub>3</sub>, 3 h, 71%; (b) Pd(OH)<sub>2</sub>/C, H<sub>2</sub>, 1 atm, overnight, 85%.



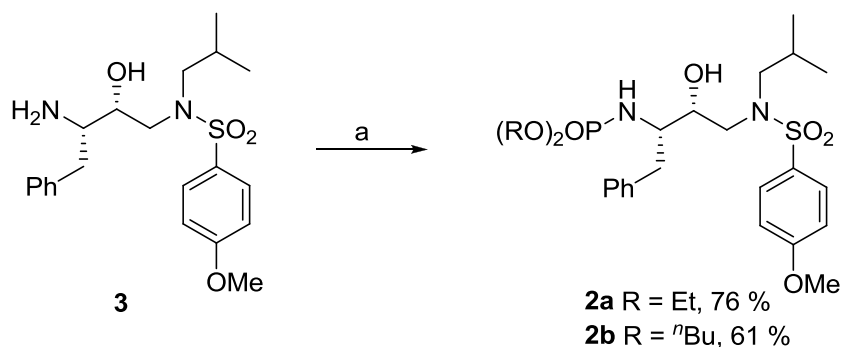
**Scheme 6.** Reagents and conditions: (a) RCHO, Diethyl phosphite, MWI, Amberlite-IR 120 (acidic).



**Scheme 7.** Reagents and conditions: (a) TBDMS-Cl, Imidazole, DCM, rt, overnight, 85%; (b) DIBAL-H, Et<sub>2</sub>O, -78 °C, 3 h; (c) NH<sub>4</sub>Br in MeOH; (d) Isobutyl amine, 3 h; (e) NaBH<sub>4</sub>, overnight, 78% in four steps; (f) TBAF, THF, 81%; (g) 4-methoxysulfonylchloride, DCM, aq. Na<sub>2</sub>CO<sub>3</sub>, 3 h, 83%; (h) Pd(OH)<sub>2</sub>/C, H<sub>2</sub>, 1 atm, overnight, 80%.



**Scheme 8.** Reagents and conditions: (a) RCHO, Diethyl phosphite, MWI, Amberlite-IR 120 (acidic).



**Scheme 9.** Reagents and conditions: (a) DEP or DBP, CCl<sub>4</sub>, K<sub>2</sub>CO<sub>3</sub>, DCM.

All the synthesized  $\alpha$ -aminophosphonate and phosphoramidate derivatives of HEA (**1a-e** and **21a-e**) and (**2a-b**), respectively were assayed for their biological activity against wild-type HIV-1 strain III<sub>B</sub> and HIV-2 strain ROD along with the double mutant strains RES056 (K103N + Y181C) according to the MTT method in MT-4 cells. The results indicate that the phosphoramidate derivative of HEA **2a** was the most active amongst all the synthesized compounds against III<sub>B</sub> and RES056 strains. Interestingly, compound **2a** was inactive against HIV-2, it was found to be active against HIV-1 and double mutant strains RES056 with IC<sub>50</sub> values of 7.77 and 7.40  $\mu$ g/ml and selectivity factors of 8 and 9, respectively. The synthesized  $\alpha$ -aminophosphonate derivatives of HEA (**1a-e** and **21a-e**) did not show any significant activity against HIV-1 (III<sub>B</sub>) as well as HIV-2 (ROD) with IC<sub>50</sub> values greater than the corresponding CC<sub>50</sub> values, rendering selectivity indexes less than 1.

### **Chapter 3: Design and Synthesis of Cysteine Protease Inhibitors**

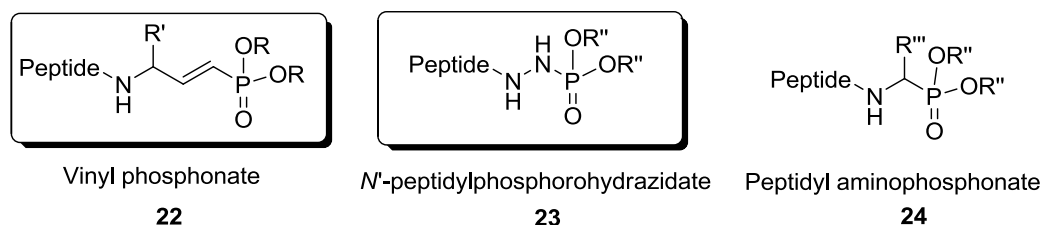
This chapter is further divided into two sections.

#### **Section A: Synthesis of Vinylaminophosphonates as Cysteine Protease Inhibitors**

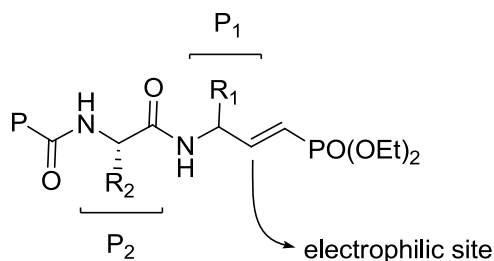
Cysteine proteases are sulfhydryl protease which catalyzes the hydrolysis of peptide, amide, ester and thiol ester bonds. Many normal physiological functions necessitate cysteine protease *e.g.* cathepsins are involved in protein breakdown in lysosomes, antigen presentation, proteolytic processing of proenzymes and prohormones, fertilization, cell proliferation, differentiation and apoptosis etc. Disharmony in the activity of cysteine protease may lead to various pathologies such as rheumatoid arthritis, multiple sclerosis, neurological disorders, tumours and osteoporosis. A molecule that can prevent the function of a protease is known as a protease inhibitor. Therefore, study directed towards design and synthesis of cysteine protease inhibitors is considerable important in the field of medicinal chemistry for the development of new drugs. Based on the mechanism, many inhibitors have been designed, synthesized and evaluated for the inhibition of cysteine proteases. Most of the inhibitor follows the usual structural scheme for development of protease inhibitors. This comprises a peptide segment for the recognition of the enzyme and an

electrophilic group which can react with the cysteine residue of the active site. Many cysteine protease inhibitors have been designed and synthesized by various research groups all over the world that includes peptidyl aldehyde, semicarbazone,  $\alpha$ -keto acids/esters, nitriles, halides and epoxides.

Based on the mechanism of proteolysis by cysteine protease, we have designed two classes of compounds *viz.* peptidyl-vinylphosphonate **22** and *N'*-peptidylphosphorohydrazidate **23** as shown in Figure 2. The designed molecules contain phosphonates or vinylphosphonates group as electrophilic centre required for the nucleophilic attack of the cysteine thiol. The peptidyl character of the designed molecule is required for the hydrophobic interaction in active site of the enzyme (Figure 3).



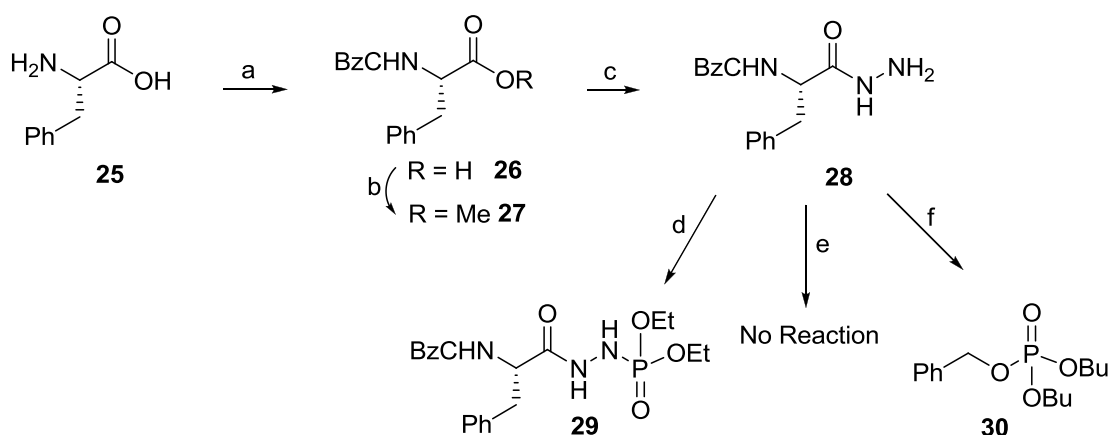
**Figure 2.** Designed cysteine protease inhibitors



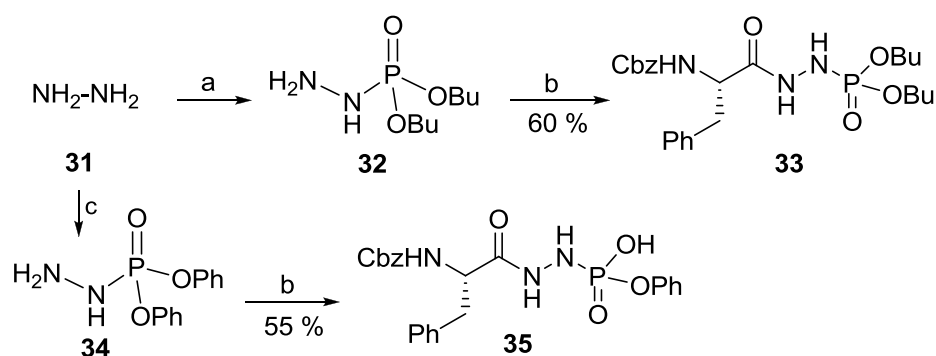
**Figure 3.** Designing of the cysteine protease inhibitors

The peptidyl aminophosphonate have been studied and are found to be inhibitors of serine protease, while peptidyl-vinylphosphonate and *N'*-peptidylphosphorohydrazidate are not studied so far for inhibition of cysteine protease.

Designed *N'*-peptidylphosphorohydrazidate have been synthesized as shown in Scheme 10 & 11.



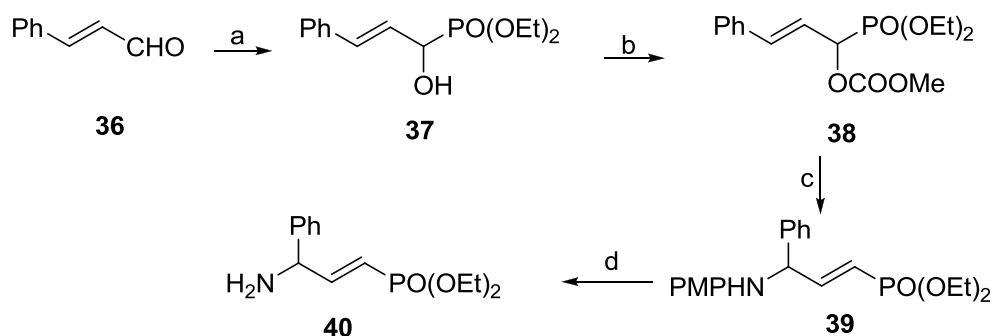
**Scheme 10.** Reagents and conditions: (a) CBz-Cl, aq. NaOH, 88%; (b) MeOH, HCl, reflux, 2 h, 93 %; (c) NH<sub>2</sub>-NH<sub>2</sub>, MeOH, rt, 1 h, 96%; (d) CCl<sub>4</sub>, diethyl phosphite, DCM, K<sub>2</sub>CO<sub>3</sub>, TBAB, 12 h, 67%; (e) CCl<sub>4</sub>, dibutyl phosphite, DCM, K<sub>2</sub>CO<sub>3</sub>, TBAB; (f) CCl<sub>4</sub>, dibutyl phosphite, DCM, aq. NaOH, 58%.



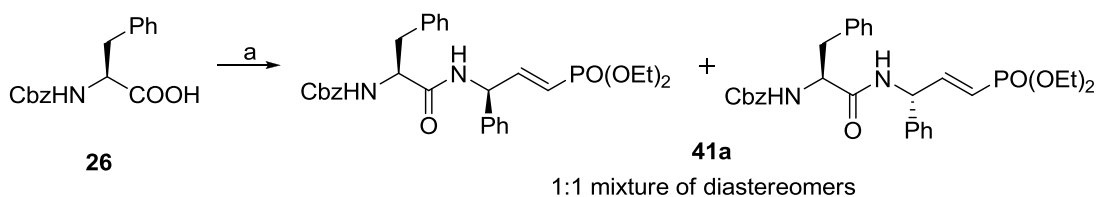
**Scheme 11.** Reagents and conditions: (a) CCl<sub>4</sub>, Dibutyl phosphite, DCM, K<sub>2</sub>CO<sub>3</sub>, TBAB, 12 h, 82%; (b) **5**, DCC, HOBT, THF, 12 h; (c) CCl<sub>4</sub>, Diphenyl phosphite, DCM, K<sub>2</sub>CO<sub>3</sub>, TBAB, 3 h, 80%.

*N'*-peptidylphosphorohydrazidates **29**, **33** and **35** were tested for *in vitro* protease inhibition activity against papain and it was observed that compound **29** inhibit protease enzyme papain with IC<sub>50</sub> value of 600 μM.

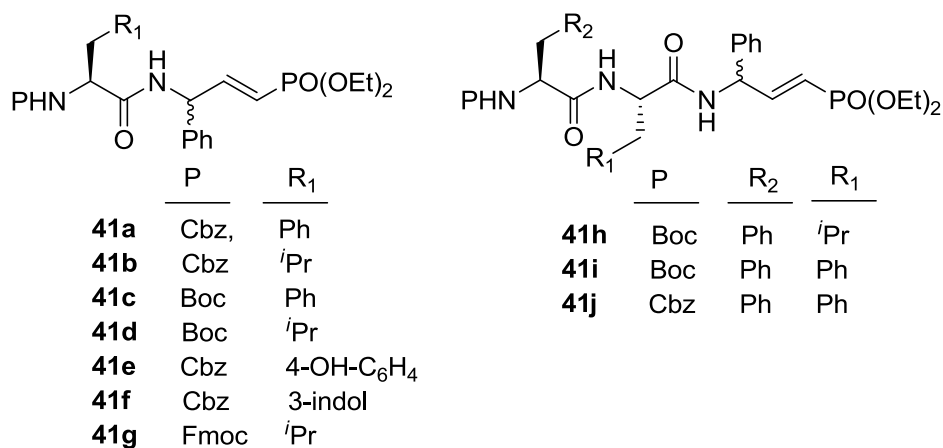
Peptidyl-vinylphosphonates were synthesized as shown in Scheme 12 and 13. Initially synthesis of vinylaminophosphonate **40** was attempted via Ritter which render final deprotection to free amine difficult. Finally **40** was synthesized using Tsuji-Trost reaction followed by PMP deprotection. Compound **40** was subjected to peptide coupling reaction to furnish peptidyl-vinylaminophosphonate **41a-j** as mixture of 1:1 diastereomers.



**Scheme 12.** Reagents and conditions: (a) DEP, TEA, 0°C-rt, 3 h, 77 %; (b) MeOCOCl, Py, MeCN, 0°C-rt, 12 h; (c) Pd(OAc)<sub>2</sub>, PMP-NH<sub>2</sub>, THF, 1 h, 95 %; (d) TCCA, MeCN, H<sub>2</sub>O, 1 M H<sub>2</sub>SO<sub>4</sub>, 12 h, 59 %



**Scheme 13.** Reagents and conditions: (a) Vinylaminophosphonate **40**, DCC, HOBT, THF 0°C-rt, 53 %.



**Figure 4.** Peptidyl vinylaminophosphonate synthesized as mixture of diastereomers (1:1).

Peptidyl vinylaminophosphonates **41a-j** were tested for *in vitro* protease inhibition activity against papain and the results are compiled in Table 1.

**Table 1.** *In vitro* protease inhibition activity of peptidyl vinylaminophosphonate

Entry	Compound	IC <sub>50</sub> (μM)	Entry	Compound	IC <sub>50</sub> (μM)
1	<b>41a (Cbz-Phe-Vp)</b>	30	6	<b>41f (Cbz-Trp-Vp)</b>	> 200
2	<b>41b (Cbz-Leu-Vp)</b>	>200	7	<b>41g (Fmoc-Leu-Vp)</b>	>200
3	<b>41c (Boc-Phe-Vp)</b>	40	8	<b>41h (Boc-Phe-Leu-vp)</b>	132
4	<b>41d (Boc-Leu-Vp)</b>	>200	9	<b>41i (Boc-Phe-Phe-Vp)</b>	83
5	<b>41e (Cbz-Tyr-Vp)</b>	54	10	<b>41j (Cbz-Phe-Phe-Vp)</b>	125

The activity data revealed that peptidyl-vinylaminophosphonates having phenylalanine in P<sub>2</sub> pocket (compounds **41a** and **41c**) were found to be more active than peptidyl-vinylaminophosphonates with leucine and other amino acids in P<sub>2</sub> pocket (compounds **41b**, **41d**, **20e** and **41f**). However, it is interesting to note that dipeptidyl-vinylaminophosphonates (**41a-g**) were more active than their corresponding tripeptidyl-vinylaminophosphonates (**41h-j**).

### **Section B: Design and Synthesis of Artemisinin-Peptidylvinylaminophosphonate Hybrid Molecules as Falcipain-2 Protease Inhibitors**

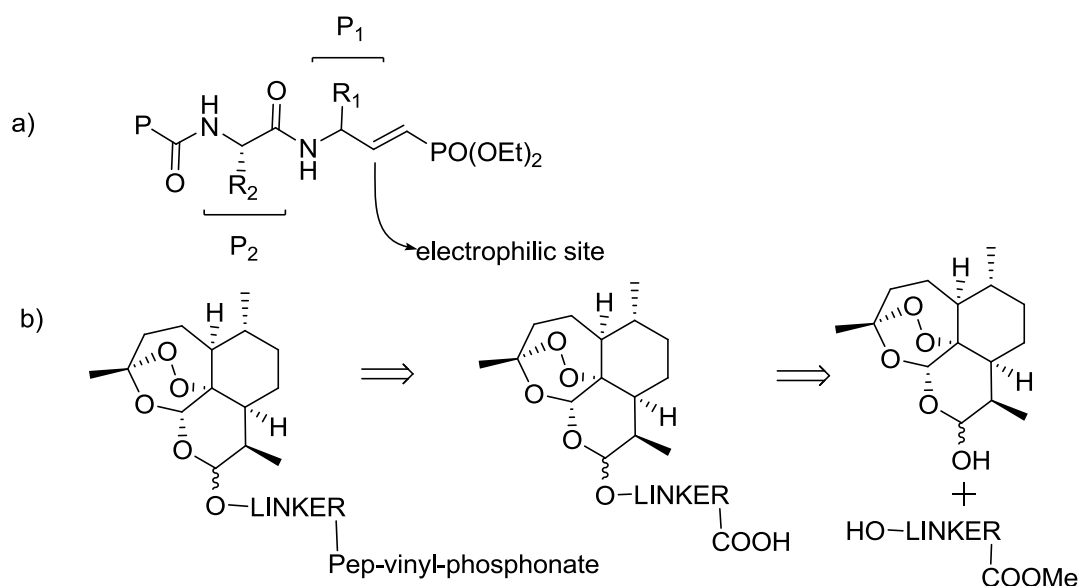
Malaria is the most common of the parasitic diseases in tropical and subtropical regions, and it is estimated that about 40% of the world's population lives in malaria endemic areas. It is caused by protozoan parasites of the genus *Plasmodium*, but in humans, it is the four species *P. falciparum*, *vivax*, *malariae* and *ovale* that are responsible for the spread of the disease. The most serious infections among these species are caused by *Plasmodium falciparum*. The currently used antimalarial drugs include: pyrimethamine, proguanil, sulfadoxine, quinine, chloroquine, primaquine, pamaquine, tefenoquine, mefloquine, atovaquone and artemisinin its semisynthetic derivatives, such as artemether and artesunate. Because of the widespread and ever increasing resistance against existing antimalarial drugs, there is increasing need for new therapeutic agents against malaria.

Artemisinin is found to be active at nanomolar concentrations *in vitro* against chloroquine-sensitive as well as chloroquine-resistant strains of *P. falciparum*. Artemisinin is one of the most important drugs for the treatment of cerebral malaria, however, its poor solubility either in oil or water, the high rate of parasite recrudescence, short-plasma half life and poor oral activity made researcher to design its derivatives which can overcome some of these shortcomings.

Hybrid drugs are considered to be formed by covalently linking two distinct chemical entities having different biological mode of action thereby creating bitherapies which would have improved biological activity and are less vulnerable to the development of drug resistance. *P. falciparum* expresses four proteases *viz.* falcipain-1 to 4. All of the falcipain are the cysteine proteases. Falcipain-2 is stored in

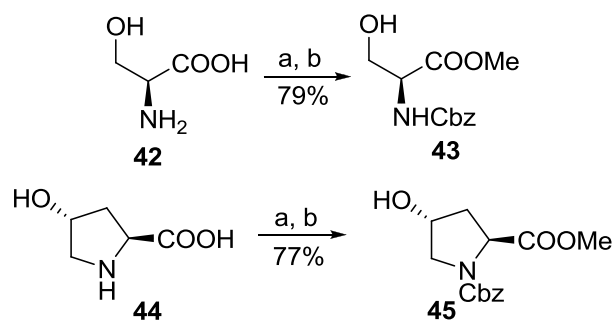


the acidic food vacuole of *P. falciparum*. It is involved in the hydrolysis of haemoglobin which produces free amino acids required for parasite. Therefore compound that inhibits falcipain-2 can also inhibit hydrolysis of haemoglobin and thereby invade parasites. Since falcipains belong to the cysteine class of protease and hence their inhibitors in principle would require hydrophobic interaction in P<sub>1</sub> and P<sub>2</sub> pockets and essentially electrophilic centre at the active-site of the enzyme. We visualized that these requirements could be ideally fulfilled by our earlier designed peptidyl-vinylaminophosphonate (Figure 5a) which were found to inhibit papain, a cysteine protease. We believe that artemisinin-peptidyl-vinylaminophosphonate hybrid molecules Figure 5b will inhibit falcipain-2 and improve the antimalarial efficacy of the artemisinin.

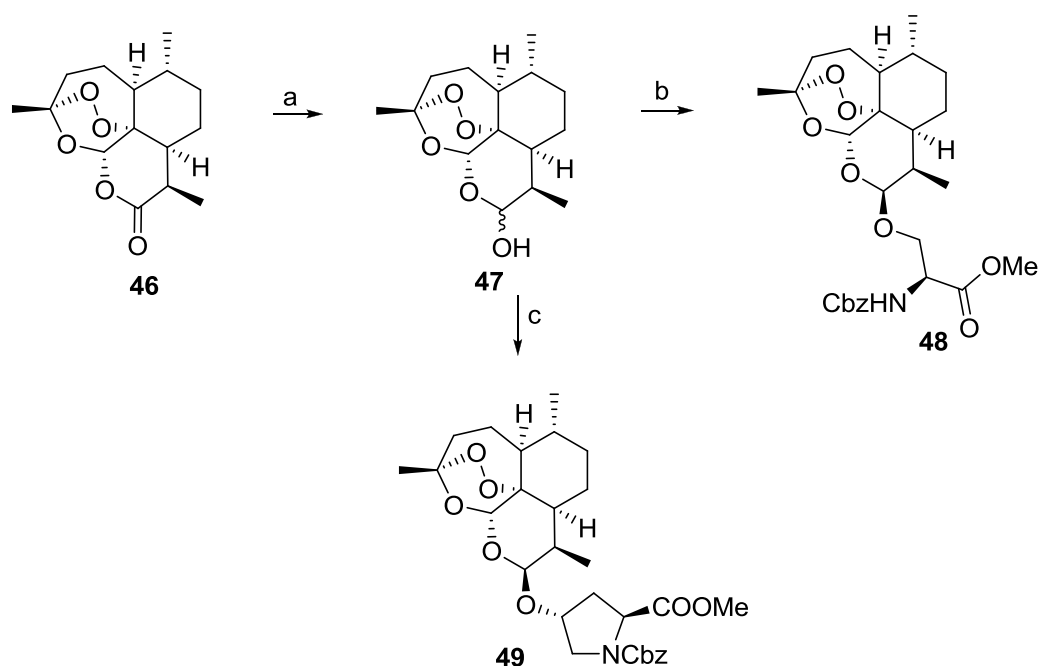


**Figure 5.** (a) Possible binding required by falcipain-2; (b) artemisinin-peptidyl-vinylaminophosphonate hybrid.

Artemisinin-peptidyl-vinylaminophosphonate hybrid molecules could be synthesized by combining dihydroartemisinin **47** with peptidyl-vinylaminophosphonate coupled through suitable linkers **43** and **45** (Scheme 14). The linker **43** and **45** when reacted with dihydroartemisinin **47** in the presence of  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  as a Lewis acid, product **48** and **49** were obtained, respectively exclusively with  $\beta$ -orientation as the only major diastereomer (Scheme 15).

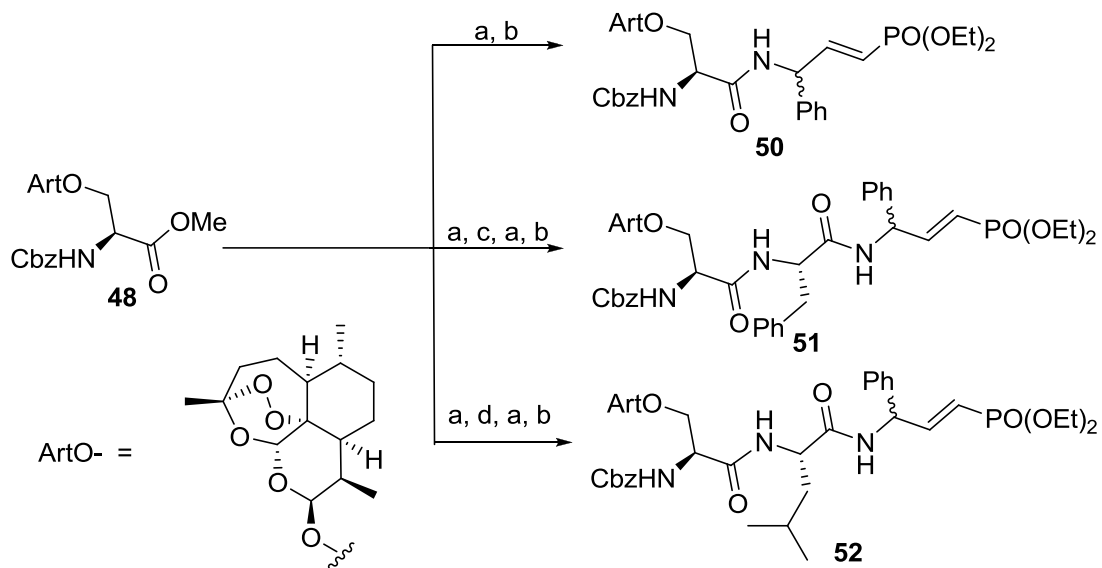


**Scheme 14.** Reagents and conditions: (a) MeCOCl, MeOH, reflux 3 h; (b) aq. NaHCO<sub>3</sub>, Cbz-Cl, rt, 3 h.

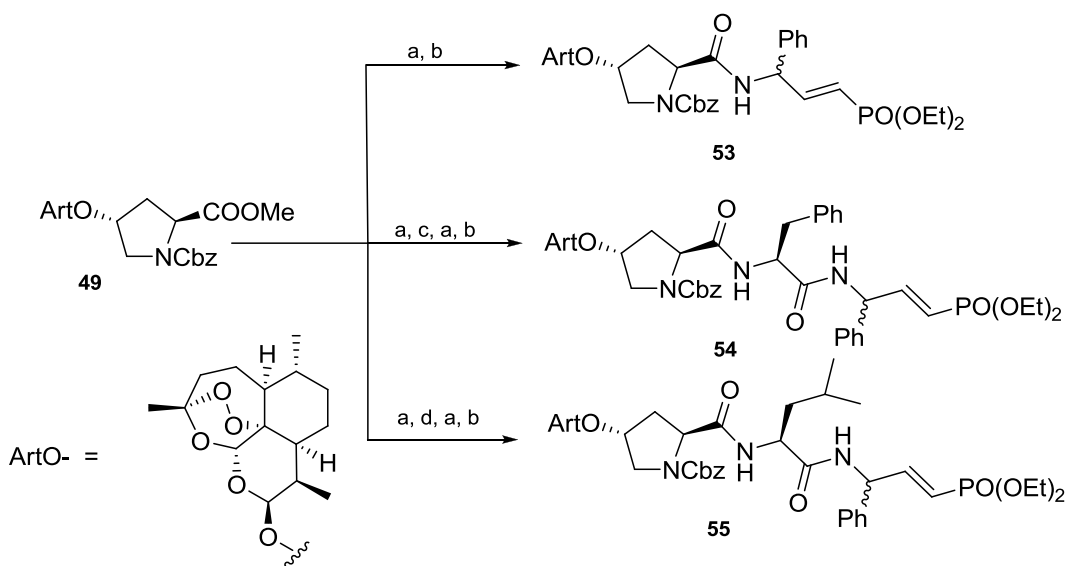


**Scheme 15.** Reagents and conditions: (a) NaBH<sub>4</sub>, MeOH, 0°C-rt, 81 %; (b) **43**, BF<sub>3</sub>·Et<sub>2</sub>O, DCM, 0°C-rt, 71 %; (c) **45**, BF<sub>3</sub>·Et<sub>2</sub>O, DCM, 0°C-rt, 67 %.

As shown in Scheme 16 and 17 compound **48** and **49** on repeated hydrolysis and peptide coupling reaction yield artemisinin-peptidyl-vinylaminophosphonate hybrids **50-55** in mixture of 1:1 diastereomers.



**Scheme 16.** Reagents and conditions: (a) LiOH, THF:H<sub>2</sub>O, rt, 2 h; (b) **40**, THF, DCC, HOBT, 0°C-rt, 3 h; (c) NH<sub>2</sub>-Phe-OMe, THF, DCC, HOBT, 0°C-rt, 3 h; (d) NH<sub>2</sub>-Leu-OMe, THF, DCC, HOBT, 0°C-rt, 3 h.



**Scheme 17.** Reagents and conditions: (a) LiOH, THF:H<sub>2</sub>O, rt, 2 h; (b) **40**, THF, DCC, HOBT, 0°C-rt, 3 h; (c) NH<sub>2</sub>-Phe-OMe, THF, DCC, HOBT, 0°C-rt, 3 h; (d) NH<sub>2</sub>-Lue-OMe, THF, DCC, HOBT, 0°C-rt, 3 h.

All the synthesized artemisinin-peptidyl-vinylaminophosphonate hybrid molecules (**50-55**) were assayed for their inhibition activity against falcipain-2 protease enzyme. The activity results of the bioassay of all the synthesized compounds are expressed in IC<sub>50</sub> values and are summarized in Table 2.

**Table 2.** *In vitro* inhibitory effects of synthesized hybrid molecules against falcipain-2

Entry	Compound	IC <sub>50</sub> Value (μM)
1	50	>100
2	51	3
3	52	4
4	53	>100
5	54	10
6	55	12

The best inhibitor of falcipain 2 among all the synthesized hybrid molecules was found to be compound **51** bearing a phenylalanine in the P<sub>2</sub> pocket and serine in P<sub>3</sub> pocket, exhibiting IC<sub>50</sub> value of 3 μM. Compound **52** was also found to be very active against falcipain-2 enzyme with IC<sub>50</sub> value of 4 μM having leucine and serine in P<sub>2</sub> and P<sub>3</sub> pocket, respectively. On the contrary, the corresponding compounds **54** and **55** having hydroxyproline in P<sub>3</sub> pocket showed IC<sub>50</sub> 10 μM and 12 μM, respectively and were found to be 3 times less active than compounds **51** and **52** having serine in the P<sub>3</sub> pocket.

#### **Chapter 4: Development of New Synthetically Useful Methodologies**

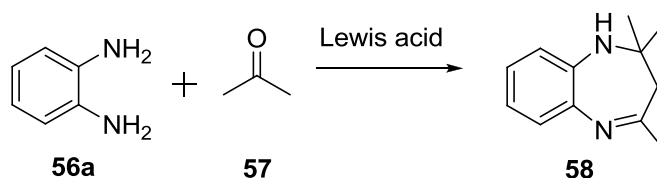
This chapter is divided into two sections.

##### **Section A: An Efficient Synthesis of Benzodiazepinylphosphonates as Clostripain Inhibitors via FeCl<sub>3</sub>-Catalyzed Four-Component Reaction**

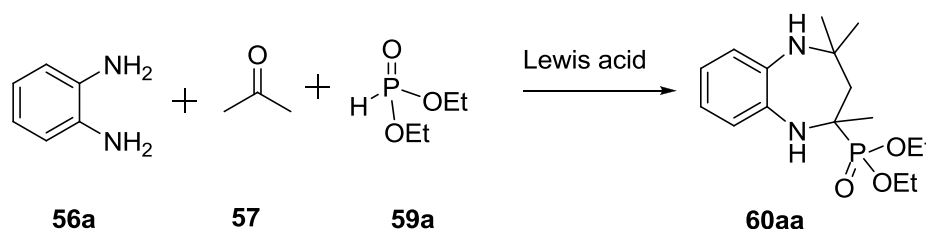
In MCR, more than two reactants are reacted in a reaction flask to furnish a product that incorporates substantial portions of all the components. In its true form, MCR involves formation of several bonds in single operation without the need for isolation of intermediates formed, changing the reaction conditions or adding further reagents. In recent years, multicomponent reactions (MCRs) have gained tremendous attention of medicinal as well as organic chemists for the generation of compound libraries of novel chemical entities to satisfy the need of high-throughput screening for new bioactive molecules having diversified scaffolds. Therefore, design and development of novel MCRs are the current need of both academia and industry. Many important name reactions are MCR in nature *e.g.* Strecker, Hantzsch, Biginelli, Mannich, Passirini, Ugi reactions etc. Some classes of compounds such as isonitrile

and 1,3-dicarbonyl compounds have found wide applications in a variety of MCRs. Similarly alkyl/aryl phosphites have also been utilized as an important participating component in some MCRs

We directed our efforts to develop a new one-pot reaction involving phosphite as one of the reactant. Since phosphites are known to attack on imine as used in Kabachnik-Field reaction, the same concept could be employed to develop a new reaction in which imine is the final product. In the literature, syntheses of benzodiazepines have been accomplished by reacting *o*-phenylenediamine and ketones catalyzed by various Lewis acids (Scheme 18). We envisaged that further nucleophilic attack of phosphite on the imine would result in the formation of BDPs in one-pot in a true MCR fashion (Scheme 19).



**Scheme 18.** Reported one-pot synthesis of benzodiazepine.

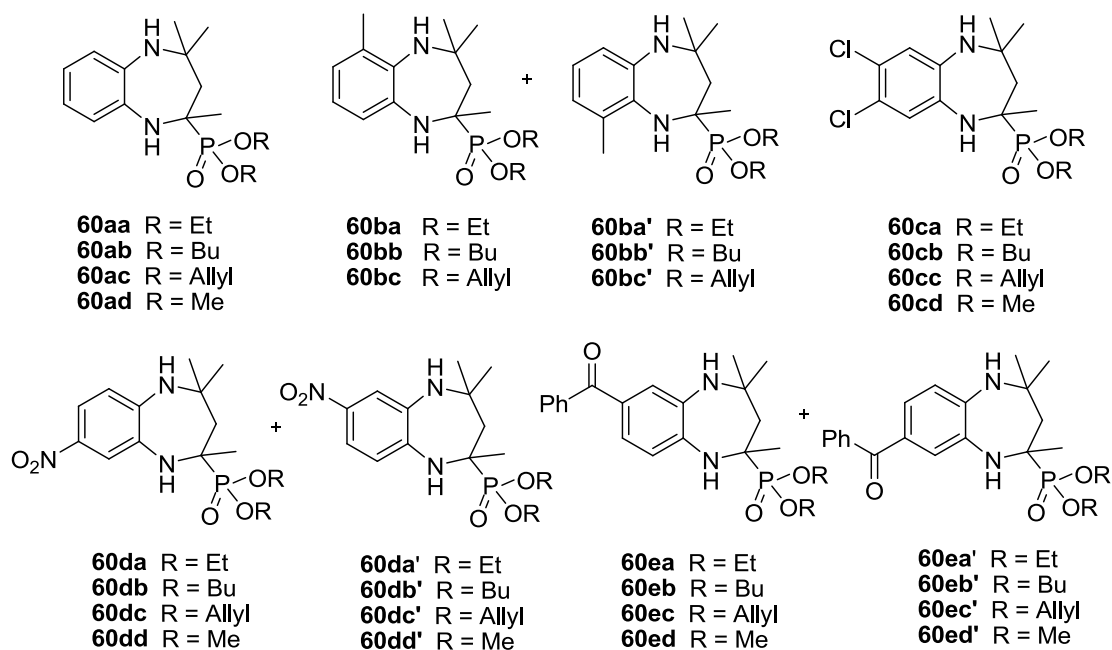


**Scheme 19.** Proposed one-pot synthesis of benzodiazepinyl phosphonate.

The benzodiazepines represent a biologically active class of compounds which exhibits wide range of therapeutic and pharmacological properties similarly  $\alpha$ -Aminophosphonates have also shown various biological activities Therefore, we opined that benzodiazepinyl phosphonates (BDPs) will be an interesting class of compounds as it combines these two biologically active moieties. Further,  $\alpha$ -aminophosphonates are considered to be the structural analogues of the corresponding  $\alpha$ -amino acids and transition-state mimics of peptide hydrolysis, the phosphonate group of  $\alpha$ -aminophosphonates can act as an electrophile which is the common requirement of cysteine protease inhibitors. This generates the possibility

that BDPs can act as cysteine protease inhibitor. Clostripain is one of the cysteine protease associated with collagenase, isolated from *Clostridium histolyticum* is an anaerobic rod-shaped, spore forming bacillus, which belongs to a group of *Clostridium* spp<sup>18</sup> causing deadly gas gangrene, a severe pathologic condition. These *clostridium* species are also responsible for various disorders like pseudomembranous colitis, food poisoning, tetanus and enteroxemia. Therefore, inhibitors of clostripain could be utilized in the therapy of gas gangrene.

Catalyst screening and reaction condition optimization revealed that FeCl<sub>3</sub> was the best catalyst and use of molecular sieves further reduced the reaction time greatly. The compounds synthesized using one pot reaction is shown in the Figure 6. In case of substituted benzene-1,2-diamine, two regioisomers were formed however, which could not be separated by repeated chromatography. Thus, ratio of the regioisomers was calculated from <sup>1</sup>H NMR.



**Figure 6.** Structures of benzodiazepinyl phosphonates synthesized through one-pot reaction

The synthesized benzodiazepinyl phosphonates were tested for their *in vitro* inhibition of clostripain and the results are summarized in Table 3. The compound **60ba+60ba'** derived from 2,3-diamino toluene and diethyl phosphite inhibited the clostripain enzyme with IC<sub>50</sub> value of 32 μM. When the phosphite was changed to allyl (**60bc+60bc'**) and butyl (**60bb+60bb'**) activity dropped to 80 and 278 μM,

respectively. Similar trend in activity profile was observed in case of benzodiazepinyl phosphonate obtained by the reaction of 3,4-diamino benzophenone. Compound **60ea+60ea'** showed IC<sub>50</sub> value of 36 μM which dropped to 70 and 140 μM in case of **60ec+60ec'** and **60eb+60eb'**, respectively.

**Table 3.** Inhibition activity of BDPs against clostripain.

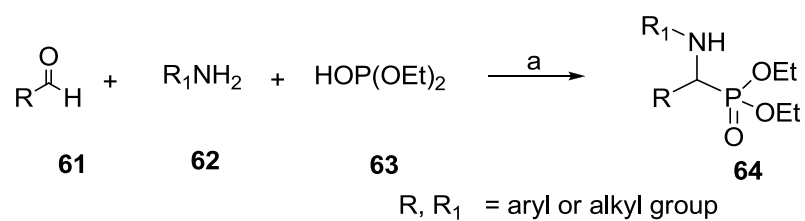
Entry	Compound Code	IC <sub>50</sub> values (μM)	Entry	Compound Code	IC <sub>50</sub> values (μM)
1	<b>60aa</b>	ND	11	<b>60cd</b>	150
2	<b>60ab</b>	780	12	<b>60da+60da'</b>	>400
3	<b>60ac</b>	>2000	13	<b>60db+60db'</b>	ND
4	<b>60ad</b>	490	14	<b>60dc+60dc'</b>	165
5	<b>60ba+60ba'</b>	32	15	<b>60dd+60dd'</b>	220
6	<b>60bb+60bb'</b>	278	16	<b>60ea+60ea'</b>	36
7	<b>60bc+60bc'</b>	80	17	<b>60eb+60eb'</b>	140
8	<b>60ca</b>	>300	18	<b>60ec+60ec'</b>	70
9	<b>60cb</b>	175	19	<b>60ed+60ed'</b>	90
10	<b>60cc</b>	>300			

The activity data revealed that many of the BDPs showed inhibition activity against clostripain. Mixture of regio-isomers **60ba+60ba'** and **60ea+60ea'** have shown good inhibition activity against clostripain with IC<sub>50</sub> value of 32 and 36 μM, respectively. Therefore, the regioisomer **60ba+60ba'** as well as **60ea+60ea'** were separated using preparative TLC. The assignment of both the regioisomers was on the basis of the HMBC correlations. The clostripain inhibition activities of these separated BDPs were further carried out. The activity profile of the separated regioisomers indicates that the major regioisomers are more active than their corresponding minor regioisomers.

### **Section B: Amberlite-IR 120 Catalyzed Three-Component Synthesis of α-Aminophosphonates in One-Pot**

We have developed a new microwave-assisted method for the synthesis of α-aminophosphonate using Amberlite-IR 120 as a solid acid catalyst (Scheme 20). Amberlite-IR 120 was found to be an efficient catalyst in one-pot reaction of aldehydes, amines, and diethyl phosphite to afford α-aminophosphonates in good to excellent yields. The main advantages of the present synthetic protocol are: mild, solvent free conditions, ecofriendly catalyst and easy reaction work-up procedure. It is

expected that the methodology will find much better application in organic synthesis due to low cost, non-toxic nature and reusability of the catalyst.



**Scheme 20.** *Reagents and conditions:* (a) Amberlite IR 120 (acidic), MWI, neat.





# **Chapter 1**

**General Introduction of Protease Inhibitors and  
Literature Review of Aminophosphonates**

## **Section A**

**General Introduction of Protease Inhibitors**

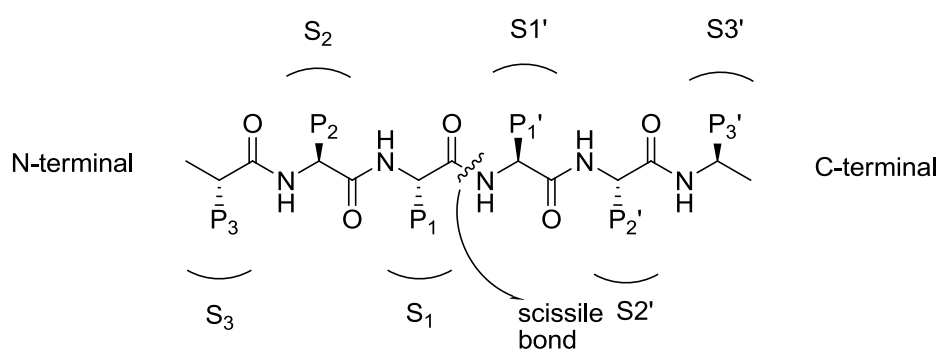
## Protease

Proteases also known as proteinase enzymes<sup>1,2</sup> catalyze the sequence selective hydrolysis of amide bond, an enzymatic reaction crucial to many physiological and pathological processes such as cell proliferation, tissue remodelling, embryonic development, blood coagulation, blood pressure control, protein activation and maturation, protein catabolism, protein transport, inflammation, infection, and cancer. Protease enzymes stabilize the transition-state of the amide hydrolysis reaction. Proteases are vital for an organism for various metabolic processes *e.g.* digestive proteases such as pepsin, is an acid protease secreted into the stomach, serine proteases (trypsin and chymotrypsin) present in duodenum enable us to digest the protein in food; proteases like thrombin, plasmin and Hageman factor, etc. play crucial role in blood-clotting and regulate action of the immune system. Many proteases are implicated in various disease *e.g.* HIV-1 protease in AIDS,<sup>1</sup> renin in blood pressure,<sup>1</sup> cathepsin D in cancer, ACE in hypertension,<sup>1</sup> cathepsin K in osteoporosis,<sup>1</sup> MMP in cancer, arthritis, fibrosis<sup>1</sup> thrombin in thrombosis and unstable angina,<sup>1</sup> elastase in rheumatoid arthritis, chronic bronchitis,<sup>1</sup> tryptase in asthma<sup>1</sup> falcipain in malaria,<sup>3</sup> clostripain in gas gangrene,<sup>4</sup> caspases in inflammatory diseases,<sup>5</sup> calpains in stroke and Alzheimer's disease<sup>6,7</sup> and NS3 protease in hepatitis C.<sup>1,8</sup> Bioinformatics analysis of the mouse and human genomes suggest that approximately 500-600 proteases (MEROPS database; <http://merops.sanger.ac.uk>) have been identified, many of which are orthologous.<sup>9</sup>

According to the catalytic mechanism, there are two broad classes of protease enzymes. In the first class of enzymes, the bounded water molecule acts as nucleophile to attack the amide carbonyl of the scissile bond. While the second class of the enzyme uses nucleophilic atom of an amino acid to initiate amide hydrolysis *e.g.* the thiol group of the cysteine residue. The nucleophilic atom of the enzyme attacks the amide carbonyl of the scissile bond and breaks the CN bond to form acyl-enzyme intermediate. This intermediate gets hydrolysed by water to complete the hydrolysis process. Protease enzyme is sequence selective. They hydrolyze a peptide bond between two selective amino acid residues. This is because the enzyme binding site which is complementary to one or more substrate residue. According to the

position of the scissile bond proteases are classified as exopeptidases *i.e.* cleaving one or a few amino acid from N- or C- terminal and endopeptidase that acts internally in polypeptide chains.<sup>1</sup>

Schechter and Berger were the first to describe the subsite nomenclature in protease system.<sup>10</sup> In this system, amino acid residues to the left of the scissile bond are numbered as P<sub>1</sub>, P<sub>2</sub> etc. numbering increases in the direction of N-terminal residue of the substrate or inhibitor while residue to the right of the scissile bond are numbered as P<sub>1</sub>' , P<sub>2</sub>' etc. The corresponding complementary regions of the enzyme active site are numbered as S<sub>1</sub>, S<sub>2</sub>, S<sub>1</sub>' etc (Figure 1).



**Figure 1.** Standard nomenclature for the substrate residue and their corresponding binding sites.

On the basis of mechanism of action of proteases they are further broadly classified as aspartic, serine, cysteine and metallo proteases.<sup>1</sup> Aspartic protease has aspartic acid in the active site of the enzyme. Similarly, serine and cysteine proteases have serine and cysteine residues in the active site of the enzyme, respectively. Metallo proteases are characterized by the presence of metals like Zn in the active site of the enzyme.

## Protease Inhibitors

Protease inhibitors are molecules that inhibit the function of proteases. Protease inhibitors prevent an undesired cleavage of a peptide or protein substrate by binding, reversibly or irreversibly to the active site of the protease.

Inhibitors of proteases are emerging with promising therapeutic uses in the treatment of diseases such as cancers, parasitic, fungal, and viral infections, malaria, HIV, hepatitis, herpes, inflammatory, immunological, respiratory, cardiovascular, and

neurodegenerative disorders including Alzheimer's disease. The protease inhibitors need to have minimal peptide character, good membrane permeability, high selectivity for a protease, high stability to non-selective proteolytic degradation, long lifetimes in the bloodstream as well as in cells, low susceptibility to elimination and good bioavailability in order to be effective drugs. These requirements are generally fulfilled by compounds having a low molecular weight (<1000 Da).<sup>1</sup>

Protease inhibitors are also classified as (i) aspartic protease, (ii) cysteine protease, (iii) serine protease and (iv) metallo protease inhibitors based on their ability to inhibit corresponding protease enzyme.

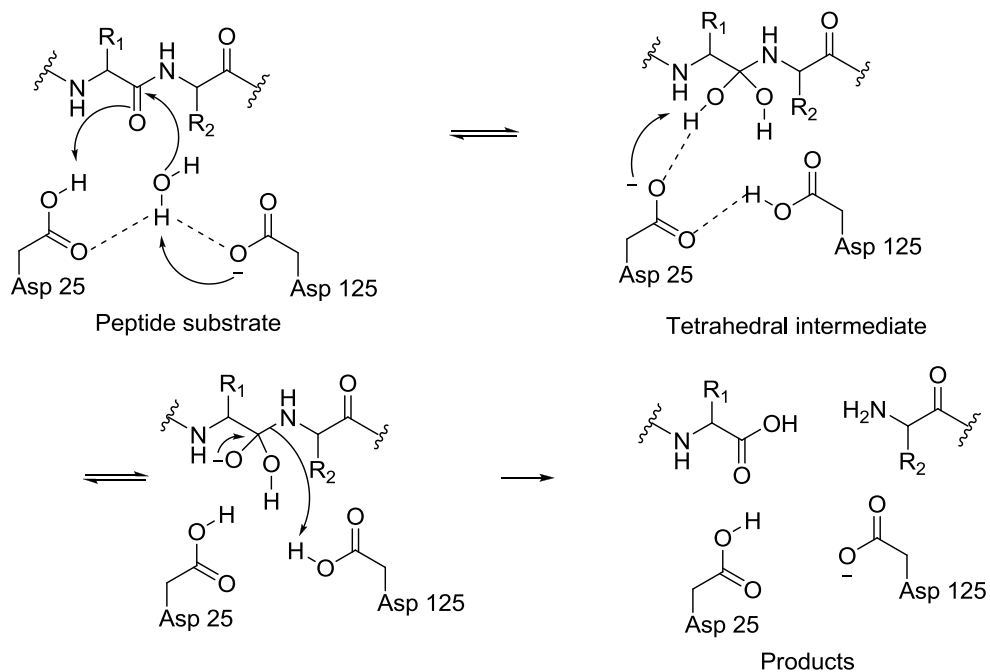
### **(i) Aspartic Protease Inhibitors**

Aspartic proteases are endopeptidases enzymes that utilize an aspartate residue for hydrolysis of their peptide substrates. They have two highly-conserved aspartates in the active site and are optimally active at acidic pH.<sup>11</sup> Aspartic proteases received enormous interest because of their significant roles in various human diseases. The best known examples are the involvement of renin in hypertension, cathepsin D in metastasis of breast cancer, the protease of human immunodeficiency virus (HIV) in acquired immune deficiency syndrome (AIDS) and plasmeprins in malaria. Designing of inhibitors of these proteases is a well known strategy to develop candidate drugs against these diseases.

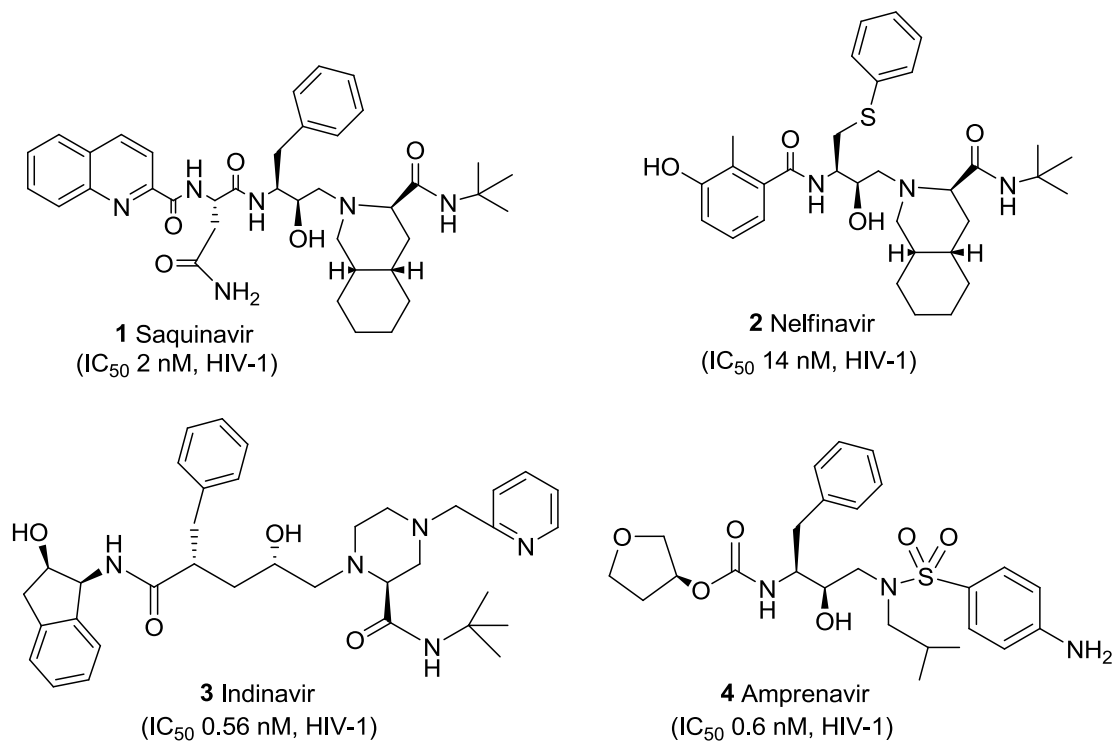
The Figure 2 describes the general acid-base mechanism which is considered for polypeptide hydrolysis catalyzed by aspartic proteases. Initially, the water molecule is partially activated by a deprotonated catalytic aspartic acid residue. The nucleophilic attack of this activated water molecule to the scissile amide bond generates the tetrahedral intermediate which collapses to form the products, acid and amine.<sup>11</sup>

Based on the mechanism and structure of the proteases, various aspartic protease inhibitors have been developed.<sup>1,12</sup> Some of them are shown in Figure 3. HIV-1 protease of human immunodeficiency virus is an attractive drug target due to its essential role in the replicative cycle of HIV. Many inhibitors of HIV-1 protease

are currently being used which includes, saquinavir **1**, ritonavir **2**, indinavir **3**, nelfinavir **4**, and amprenavir **5**. Many other aspartic protease inhibitors have also been developed such as **6** (Cathepsin D), **7** (renin) and **8** (plasmepsin I).<sup>1,12</sup>



**Figure 2.** Catalytic mechanism for substrate hydrolysis by aspartic proteases.



**Figure 3.** Aspartic protease inhibitors

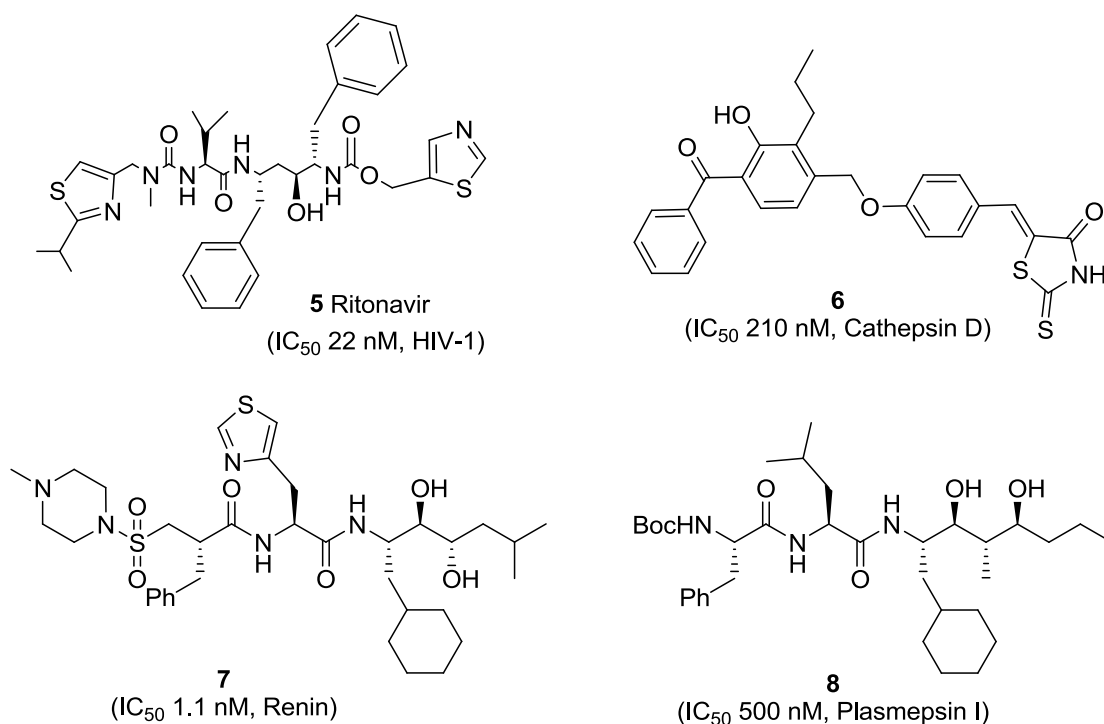


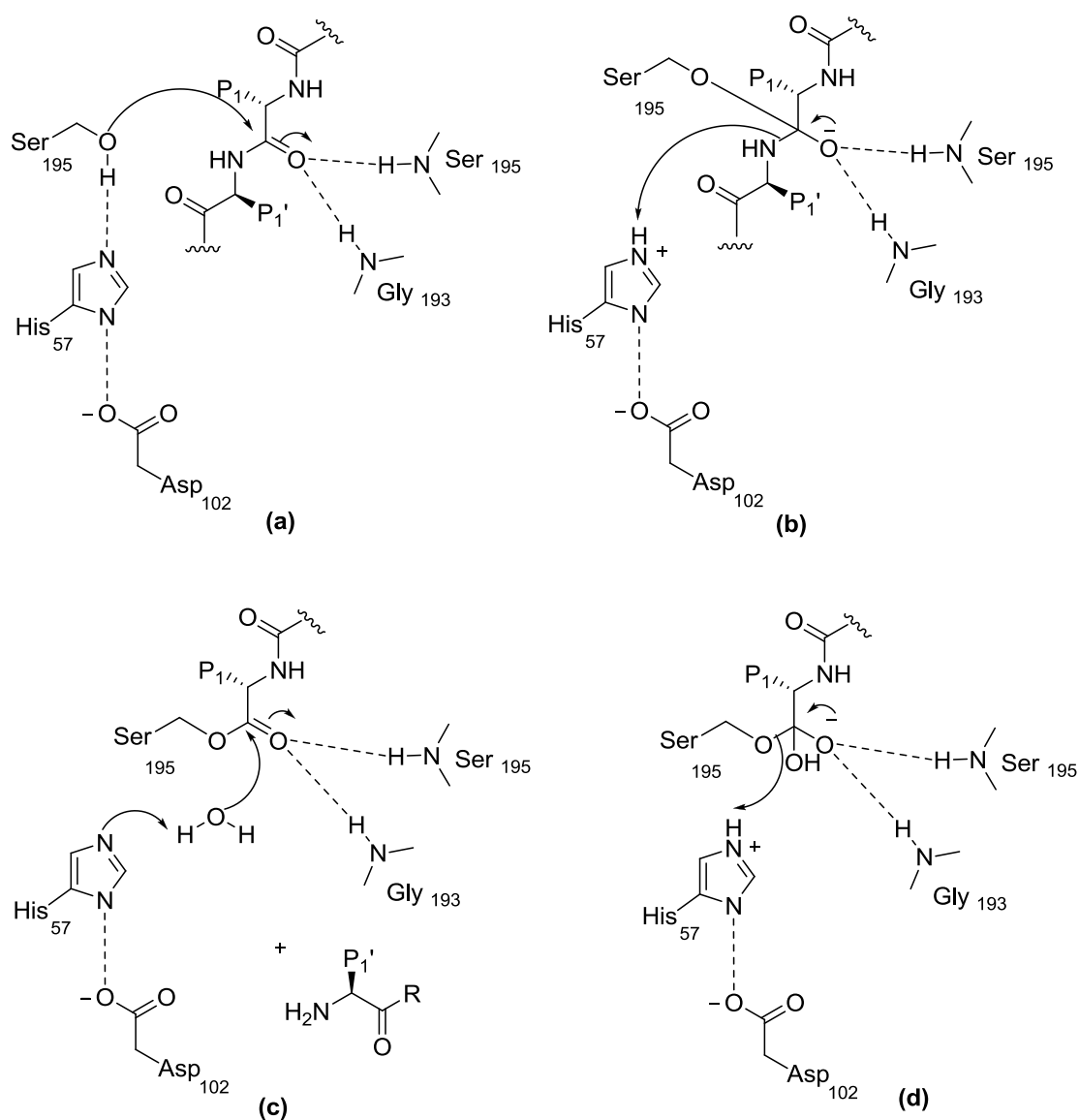
Figure 3. Aspartic protease inhibitors (continued)

## (ii) Serine Protease Inhibitors

Serine proteases are endopeptidases in which one of the amino acids in the active site of the enzyme is serine. Serine proteases are also involved in many important physiological processes in humans *e.g.* blood coagulation, immune response and digestion. Serine proteases are implicated in various diseases and disorders *e.g.* thrombin in thrombosis and unstable angina, elastase in rheumatoid arthritis, chronic bronchitis, tryptase in asthma and NS3 protease in hepatitis C.<sup>1</sup>

The active site of serine protease consists of a catalytic triad of Ser195, His57, and Asp102 residues and an oxyanion hole.<sup>1</sup> The substrate binds in the active site and the carbonyl group of the scissile amide bond is exposed to nucleophilic attack by the active site serine hydroxyl under base catalysis by the imidazole side chain of His57 (Figure 4a). The resulting tetrahedral intermediate is stabilized by hydrogen bonding to the backbone NH of Ser195 and Gly193, which form the oxyanion hole. Proton transfer from His57 to the amine of the tetrahedral intermediate facilitates expulsion of the amine fragment as leaving group (Figure 4b). The covalent acyl-enzyme

complex is attacked by water, with formation of a new tetrahedral intermediate (Figure 4c) which subsequently breaks down *via* acid-assisted catalysis by His57 to form the carboxyl fragment of the cleaved substrate and regenerate Ser195 (Figure 4d). Several serine protease inhibitors have been developed (Figure 5).<sup>1,13</sup>



**Figure 4.** General catalytic mechanism of serine protease.

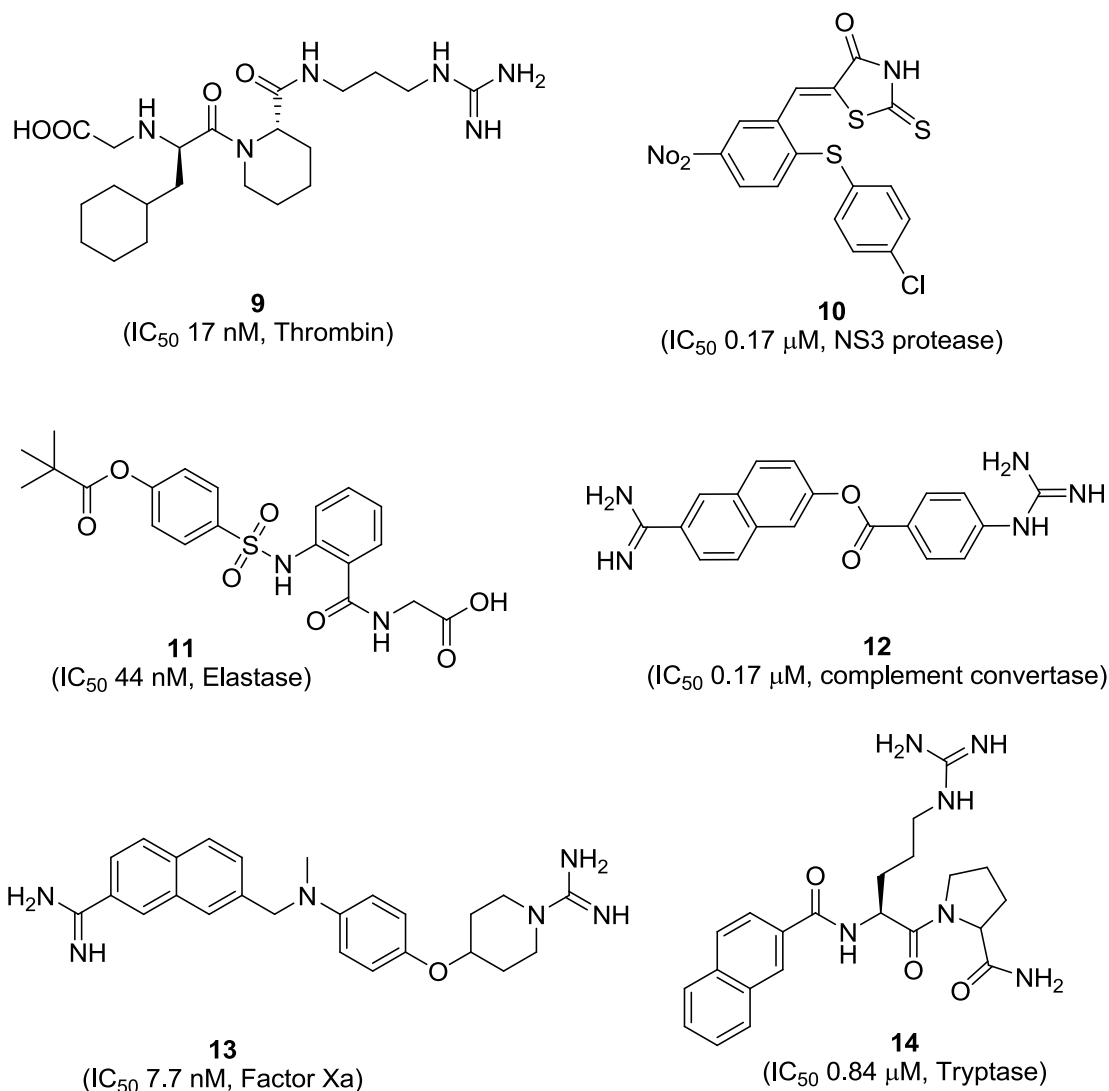


Figure 5. Serine protease inhibitors

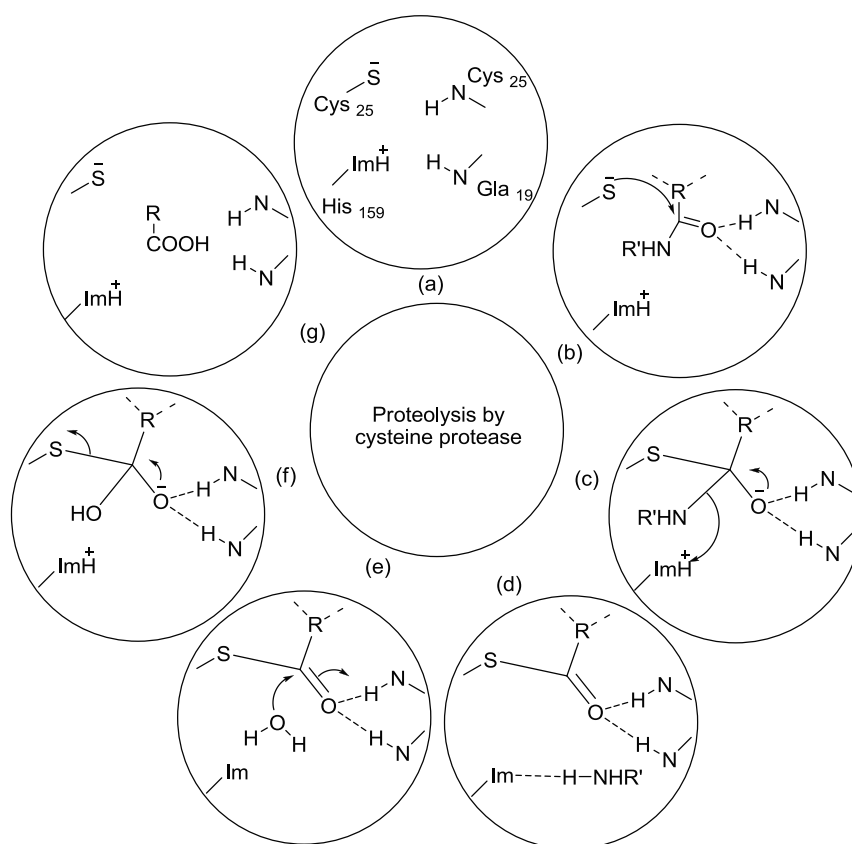
### (iii) Cysteine Protease Inhibitors

Cysteine proteases catalyze the hydrolysis of peptide, amide, ester and thiol ester bonds by using nucleophilic cysteine thiol in the active site. They are essential to many normal physiological functions *e.g.* cathepsins are involved in protein breakdown in lysosomes, antigen presentation, proteolytic processing of proenzymes and prohormones, fertilization, cell proliferation, differentiation and apoptosis etc. and are implicated in various diseases such as rheumatoid arthritis, multiple sclerosis, neurological disorders, tumors, osteoporosis asthma calpain in Alzheimer's disease, muscular dystrophy, type 2 diabetes, traumatic brain and spinal cord injury and cerebral ischemia, gingipain in gingivitis, caspases in neurodegenerative disorders,



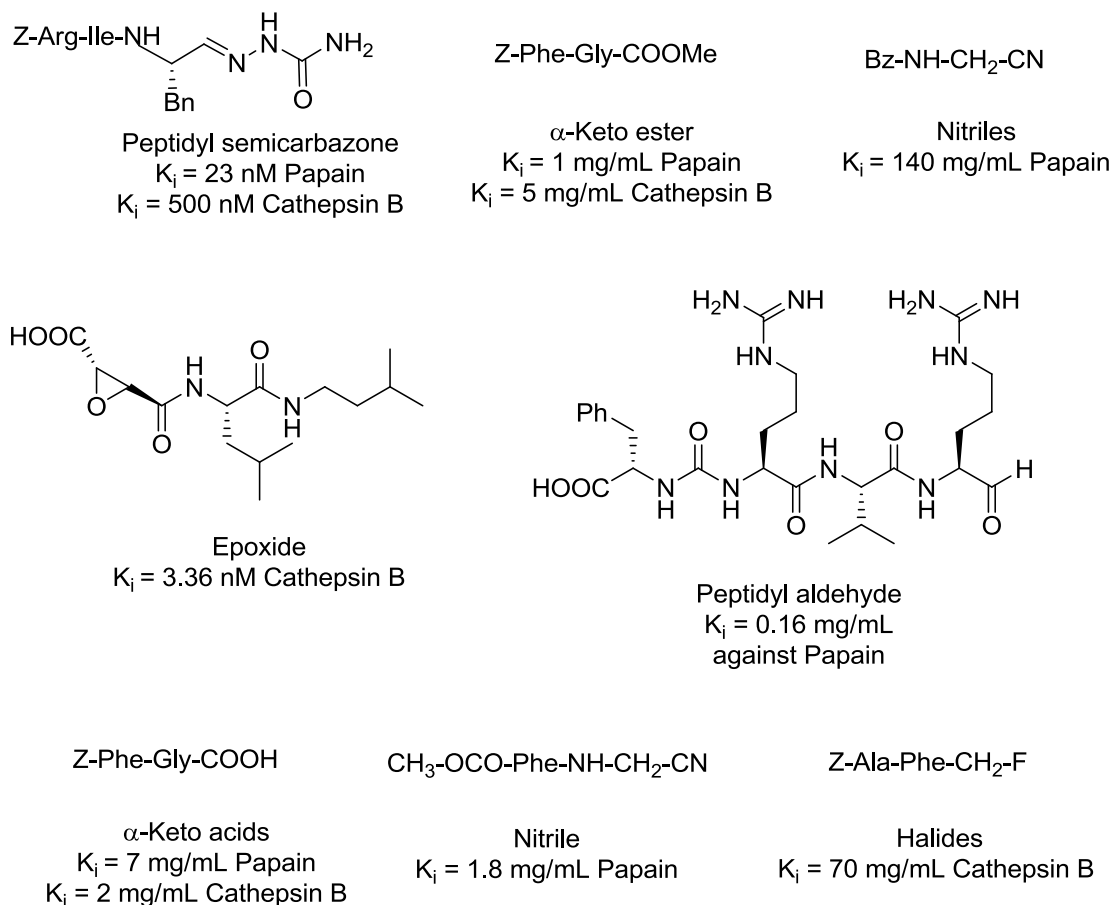
legumain in parasitic infections, separase in cancer and clostripain in bacterial infections.<sup>1,14-20</sup>

The general mechanism of cysteine protease has been very well studied with papain as the model enzyme. The thiol of the cysteine (Cys) is polarized by the imidazole of the Histidine (His) allowing for deprotonation (Figure 6a).<sup>21</sup> The substrate is non-covalently bound into the active site *via* hydrogen bonds of the carbonyl forming the enzyme-substrate complex (Figure 6b). The catalytic cysteine thiol then attacks the carbonyl forming a tetrahedral intermediate as depicted in Figure 6c. The imidazole of the catalytic His then acts as a general acid protonating the leaving group of the substrate resulting in the formation of acyl intermediate (Figure 6d) followed by the abstraction of a proton from a water molecule by catalytic His (Figure 6e). The activated water molecule then hydrolyzes the thioester of the acyl intermediate bond (Figure 6f) resulting in the formation of free acid (Figure 6g) and enzyme active site gets free for further hydrolysis (Figure 6a).



**Figure 6.** Proteolysis by cysteine protease

Based on the proteolytic mechanism exhibited by the cysteine proteases, several inhibitors have been designed, synthesized and evaluated for their inhibition which includes peptidyl aldehyde, semicarbazone,  $\alpha$ -keto acids/esters, nitriles, halides and epoxides (Figure 7).<sup>1,14,22</sup>



**Figure 7.** Known cysteine protease inhibitors.

#### (iv) Metallo Protease Inhibitors

Metalloproteases as the name suggests involve a metal *e.g.* zinc atom in the active site to affect amide bond hydrolysis. Angiotensin converting enzyme (ACE), inhibition of which represents one of the most clinically effective means to lower blood pressure, endothelin-converting enzyme (ECE) is a therapeutic target in the cardiovascular area and matrix metalloproteinases (MMP) which are implicated in various diseases like cancer, arthritis, stroke and angiogenesis are the examples of the metallo proteases.<sup>1</sup>

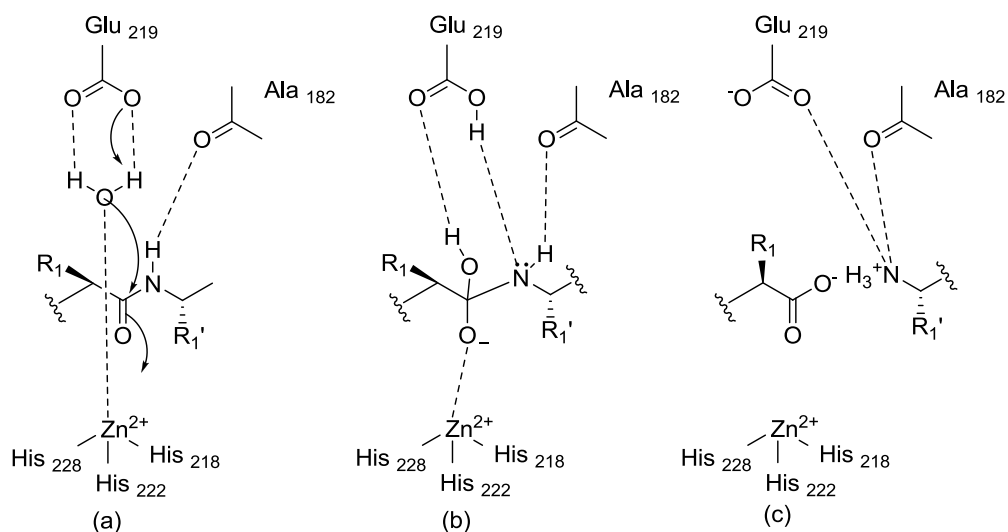


Figure 8. Catalytic mechanism for MMPs

In the active site of the MMP,<sup>23</sup> the  $Zn^{2+}$  ion is tetrahedrally coordinated to three donor groups from the enzyme and a water molecule. The water is also hydrogen-bonded to the carboxylate side chain of a glutamic acid and is therefore, activated for nucleophilic attack (Figure 8a). Zinc-complexed tetrahedral intermediate is formed by simultaneous nucleophilic attack of the zinc bound water and proton transfer to the carboxylate (Figure 8b). Transfer of a proton from the glutamic acid to the amide nitrogen is followed by the collapse of the tetrahedral intermediate with the generation of a salt bridge between glutamic acid and free amine of the cleaved substrate (Figure 8c). Several metallo protease inhibitors have also been designed and synthesized (Figure 9).<sup>23</sup>

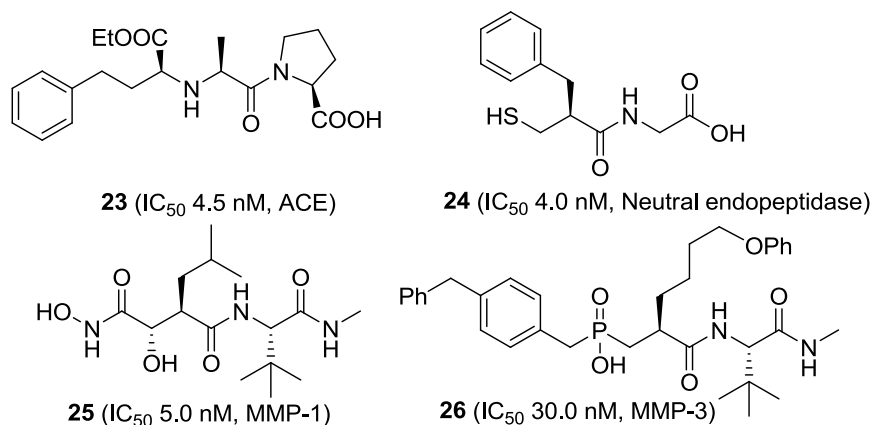


Figure 9. Metallo protease inhibitors.

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# **Chapter 1**

**General Introduction of Protease Inhibitors and  
Literature Review of Aminophosphonates**

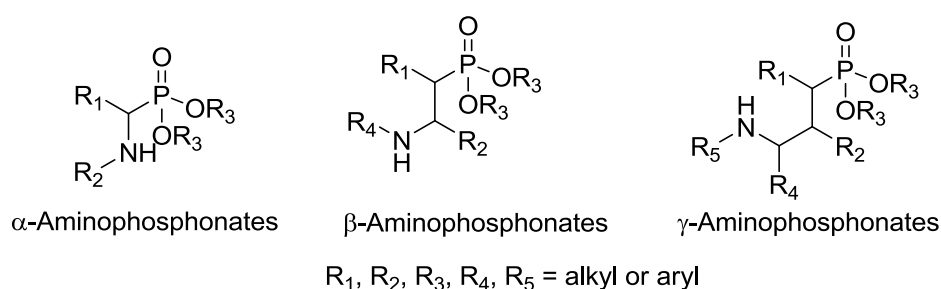
## **Section B**

**Literature Review of Aminophosphonates**



## Introduction

Aminophosphonates (Figure 1) are the chemical compounds that possess amino and phosphonate functionalities. Aminophosphonates could be further classified into three major classes *viz.* (i)  $\alpha$ -aminophosphonate, (ii)  $\beta$ -aminophosphonates and (iii)  $\gamma$ -aminophosphonates.



**Figure 1.** Generalize structure of aminophosphonates.

Due to the tetrahedral configuration of phosphorus which has important implications in the design transition-state analogue enzyme inhibitors, hence aminophosphonates also show various biological activities.<sup>1-6</sup>

### (i) $\alpha$ -Aminophosphonates

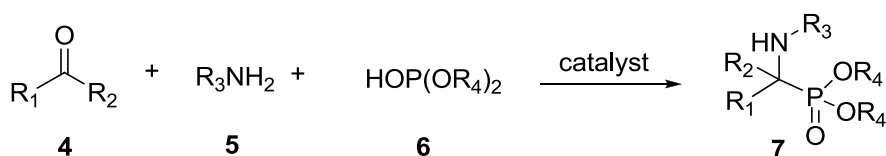
$\alpha$ -Aminophosphonates are considered to be structural analogues of ester of  $\alpha$ -amino acids and therefore, find immense importance in the biology.<sup>1</sup> With respect to shape, size, and acidity, phosphonic and carboxylic acid groups are considerably different. Among the various compounds containing a P–C bond, aminophosphonates established an important place because they are analogues of natural  $\alpha$ -amino acids, which are the ‘building blocks’ of peptides and proteins. The replacement of acid group by phosphonic acid group have attracted particular interest and have reached a position of eminence in fields of research directed to the discovery, understanding, and modification of physiological processes in living organisms.<sup>1</sup>

### Synthesis of $\alpha$ -Aminophosphonate

Various methods of synthesis of  $\alpha$ -aminophosphonates have been developed and some of these are discussed briefly.

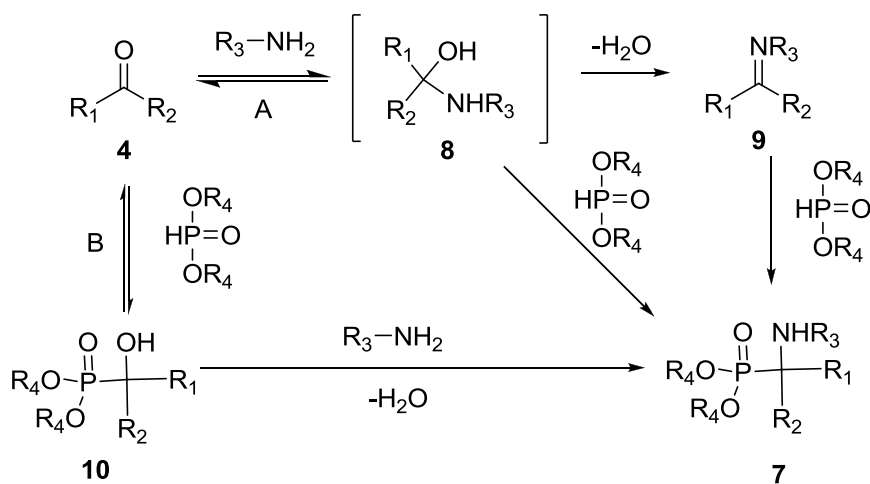
## 1. Kabachnik-Field reaction

In 1952 Martin Izrailevich Kabachnik<sup>7</sup> and Ellis K. Fields<sup>8</sup> had discovered a novel multicomponent reaction for the synthesis of  $\alpha$ -aminophosphonate from an amine, a carbonyl compound and a dialkyl phosphite. The multicomponent reaction was named after them as Kabachnik-Field reaction (Scheme 1). This reaction is very useful from the combinatorial chemistry point of view.<sup>9</sup>



**Scheme 1.** Kabachnik-Field Reaction: synthesis of  $\alpha$ -aminophosphonates

The reaction of condensation of aldehyde ( $\text{R}_1=\text{H}$ ), amine and phosphite is satisfactory however only few examples are reported for the Kabachnik-Fields reactions with ketones instead of aldehyde.<sup>10</sup>



**Scheme 2.** Mechanism of Kabachnik-Field reaction.

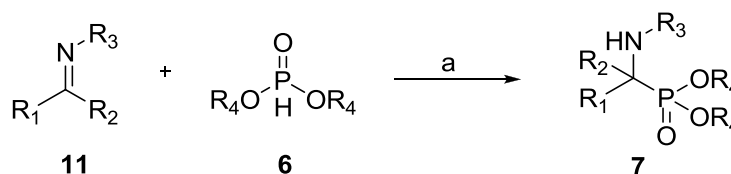
It is obvious that the first step is the formation of the corresponding imine **9** (Pathway A) through intermediate **8** which is a reversible process. Therefore, removal of the water formed by using dehydrating agents or molecular sieves is helpful for the success of the reaction. In the second step, phosphite adds to the C=N bond of the imine **9** to give phosphonates **7** (Scheme 2).<sup>11</sup> Phosphite can also directly substitute the hydroxyl group of the intermediate **8** to furnish **7**. The other plausible mechanism involve the addition of the dialkyl phosphites to C=O bond giving  $\alpha$ -

hydroxyphosphonates **10** (Scheme 2, path **B**) which can also lead to the product **7** of Kabachnik-Fields reaction by the direct nucleophilic substitution of the hydroxyl in  $\alpha$ -hydroxyphosphonates by amino component.<sup>12</sup>

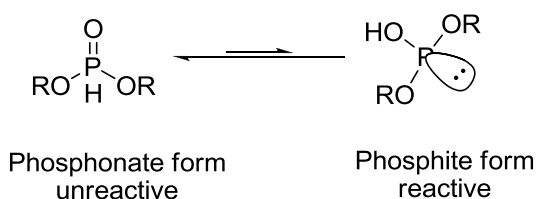
Conventionally, the reaction is carried out by mixing equimolar quantities of all components in the appropriate solvent with or without removal of water by azeotropic distillation. However, in recent times use of catalysts with dehydrating agent or water tolerant catalysts, for example, some rare earth metal triflates have gained use in the Kabachnik-Field reactions. Owing to the great biological importance of  $\alpha$ -aminophosphonates, various catalytic systems have been studied for this reaction which includes tetramethylguanidine,<sup>13a</sup> dodecatungstophosphoric acid,<sup>13b</sup> silica-gel,<sup>13c</sup> magnesium perchlorate,<sup>10e</sup> TiCl<sub>4</sub>,<sup>13d</sup> FeCl<sub>3</sub>,<sup>13e</sup> YbCl<sub>3</sub>,<sup>13f</sup> [bmim]Cl-AlCl<sub>3</sub> ionic liquid,<sup>13g</sup> montmorillonite K10,<sup>10b</sup> bismuth(III) chloride,<sup>13h</sup> gallium triiodide,<sup>13i</sup> LiClO<sub>4</sub>,<sup>13j</sup> CAN,<sup>10d</sup> samarium diiodide,<sup>13k</sup> CF<sub>3</sub>COOH,<sup>13l</sup> TaCl<sub>5</sub>-SiO<sub>2</sub>,<sup>10c</sup> indium(III) chloride<sup>10a</sup> and zirconium(IV) compounds,<sup>10f</sup> Bi(NO<sub>3</sub>)<sub>3</sub>.5H<sub>2</sub>O,<sup>10g</sup> Amberlite IR-120 (acidic)<sup>10h</sup> etc.

## 2. Hydrophosphynylation of imine

$\alpha$ -Aminophosphonates are generally synthesized by the addition of phosphites to imines (Scheme 3) known as hydrophosphynylation reaction.<sup>14</sup> In this reaction, phosphonates have been used as P-nucleophiles. Reactivity of the phosphite is due to the phosphonate-phosphite tautomerism, which exists with the phosphite form as the active nucleophilic species and the phosphonate tautomer as the almost exclusively favoured but non-nucleophilic<sup>14</sup> (Scheme 4). Hydrophosphynylation of imine is referred as Pudovik reaction. It is a two step protocol for the synthesis of  $\alpha$ -aminophosphonates *viz.* formation of imine followed by hydrophosphynylation. This reaction is either catalyzed by base or acid.



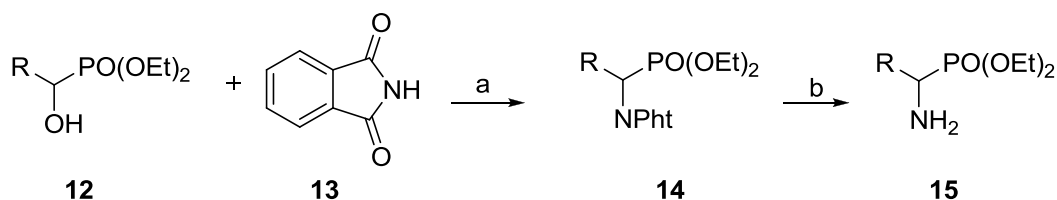
**Scheme 3.** Reagents and conditions: (a) acid or base



**Scheme 4.** Phosphonate-phosphite tautomerism

### 3. Mitsunobu reaction

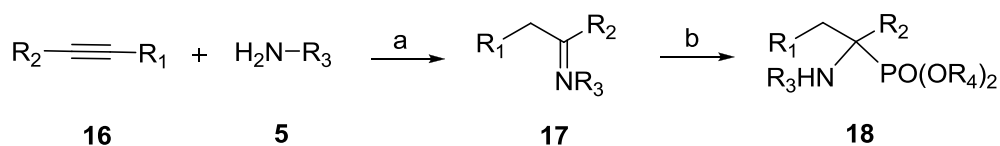
Another method for the synthesis of  $\alpha$ -aminophosphonates is the utilization of the Mitsunobu reaction.<sup>15</sup> In this method, hydroxyphosphonate is subjected to Mitsunobu reaction condition with amine like phthalimide. Finally  $\alpha$ -aminophosphonates are obtained by the deprotection of the phthalimide group (Scheme 5).



**Scheme 5.** Reagents and conditions: (a)  $\text{Ph}_3\text{P}$ , DEAD, THF; (b) Hydrazine hydrate.

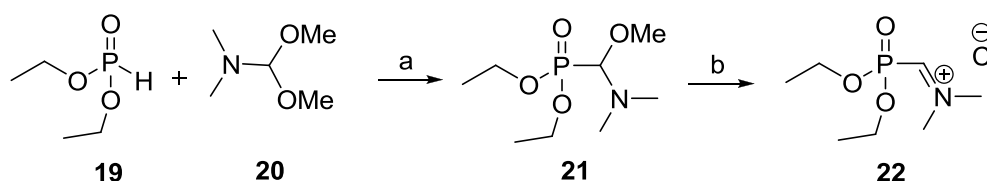
### 4. Other methods

Doye *et al*<sup>16</sup> reported a novel procedure for the synthesis of  $\alpha$ -aminophosphonates by employing alkynes, primary amines and diethyl or dimethyl phosphites. The reaction is a one-pot operation. Initially  $\text{Cp}_2\text{TiMe}_2$  catalyzed hydroamination of the alkyne takes place which is followed by subsequent nucleophilic addition of diethyl or dimethyl phosphite to the resulting imine, performed in the presence of catalytic amount of  $\text{Me}_2\text{AlCl}$  furnishes the desired  $\alpha$ -aminophosphonates (Scheme 6).

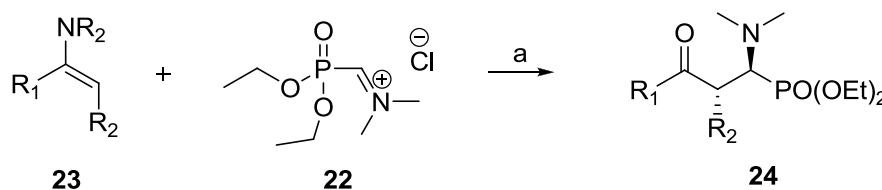


**Scheme 6.** Reagents and conditions: (a) 3.0-5.0 mol%,  $\text{Cp}_2\text{TiMe}_2$ , 110 °C; (b)  $\text{HPO}(\text{OR}_4)_2$ , 5.0 mol%,  $\text{Me}_2\text{AlCl}$ , 25 °C

Risch *et al* reported<sup>17</sup> the synthesis of  $\alpha$ -aminophosphonates using phosphonate substituted iminium salt **22** in Mannich reactions with nucleophiles like enamine **23** to obtain novel  $\alpha$ -aminophosphonates **24**. Initially diethyl phosphonate and DMF dimethyl acetal **20** were reacted followed by the addition of thionyl chloride to furnish the C-phosphoryl aldiminium salt **22** (Scheme 7). The enamine **23** was subjected to aminoalkylation under mild conditions to afford the desired  $\alpha$ -aminophosphonates **24** (Scheme 8). The reaction proceeds with very high diastereoselectivity and only one diastereoisomer was observed by NMR spectroscopy.

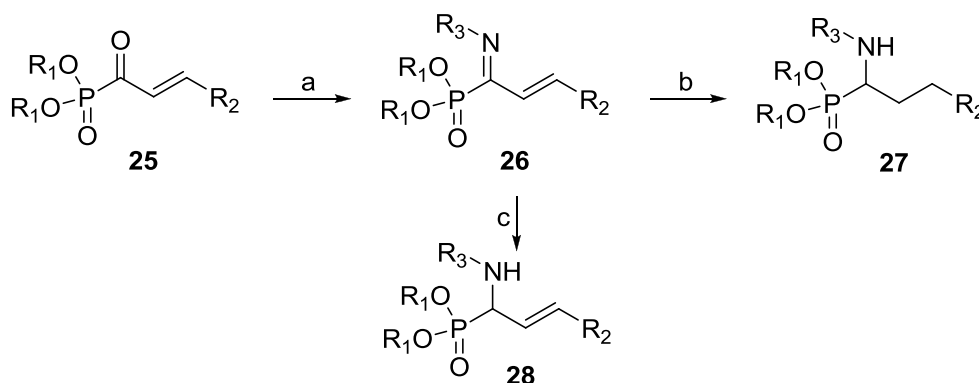


**Scheme 7.** Reagents and conditions: (a) neat, rt; (b)  $\text{SOCl}_2$ ,  $\text{Et}_2\text{O}$ ,  $0^\circ\text{C}$ , 30 min.



**Scheme 8.** Reagents and conditions: (a) DCM,  $-80$  to  $-30^\circ\text{C}$ , 15 h; aq. HCl.

Palacios *et al*<sup>18</sup> had reported synthesis of  $\alpha$ -aminophosphonates from  $\beta,\gamma$ -unsaturated  $\alpha$ -ketophosphonates **25**. This novel approach utilized aza-Wittig reaction of trimethyl phosphazenes followed by hydrogenation to furnish  $\alpha$ -aminophosphonates **27** or **28** depending on the hydrogenation conditions (Scheme 9).



**Scheme 9.** Reagents and conditions: (a)  $\text{PMe}_3$ , toluene,  $0^\circ\text{C}$ ; (b)  $\text{H}_2$ , Pd/C, 80 psi, rt; (c)  $\text{BH}_3\cdot\text{SMe}_2$ ,  $-78^\circ\text{C}$ .

## Asymmetric synthesis of $\alpha$ -aminophosphonates

Asymmetric synthesis of  $\alpha$ -aminophosphonates<sup>19</sup> has garnered tremendous interest from various research groups in recent times. The asymmetric synthesis of  $\alpha$ -aminophosphonates could be carried out in two different ways, *e.g.*

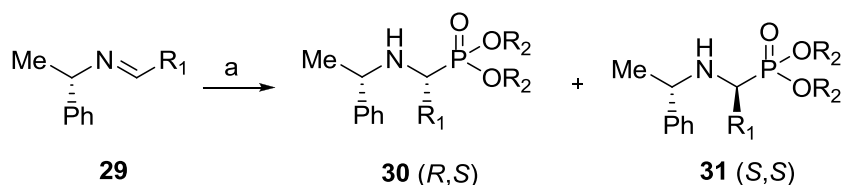
- (i) Chiral pool approach or
- (ii) Catalytic asymmetric synthesis

### (i) Chiral Pool approach

Chiral pool approach involves synthesis of  $\alpha$ -aminophosphonates using chiral starting materials *i.e.* carbonyl compounds or amines or phosphites. The chiral pool synthesis of  $\alpha$ -aminophosphonates has been well documented in literature.<sup>19</sup>

#### (a) Using chiral amines

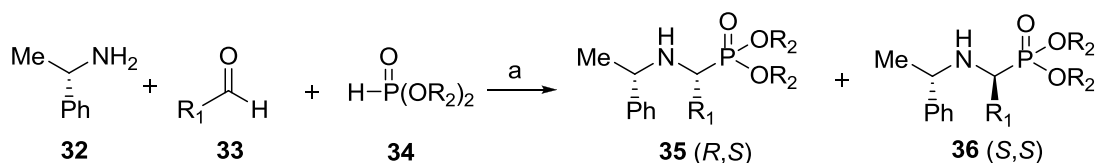
Many different chiral amines have been used for the asymmetric synthesis of  $\alpha$ -aminophosphonates.<sup>19,20,10a</sup> The chiral amines used are (*S*)- $\alpha$ -methylbenzylamine, (*R*)- $\alpha$ -methylbenzylamine, esters of amino acids, (*R/S*)-phenylglycinol, chiral sulfinimides, (*R/S*)-1-(1-naphthyl) ethylamine, chiral amino alcohols etc. These amines could be used for the asymmetric one pot synthesis of  $\alpha$ -aminophosphonates *via* Kabachnik-Field reaction or in two steps *via* Pudovik reaction. Thompson *et al*<sup>20</sup> reported asymmetric synthesis of  $\alpha$ -aminophosphonothionates (*R,S*)-**30** and (*S,S*)-**31** by following Pudovik reaction conditions that is the addition of sodium salt of dialkylphosphite to the chiral imine **29**. Compounds (*R,S*)-**30** and (*S,S*)-**31** were formed in 64-98% yields with a diastereoisomeric ratio up to 97.5:2.5 (Scheme 10).<sup>20a</sup>



**Scheme 10.** Reagents and conditions: (a) sodium salt of dialkylphosphite, Yield 64-98%; dr up to 97.5:2.5

Asymmetric synthesis of  $\alpha$ -aminophosphonates (*R,S*)-**35** and (*S,S*)-**36** by employing three-component reaction of benzaldehyde, (*S*)- $\alpha$ -MBA, and diethyl

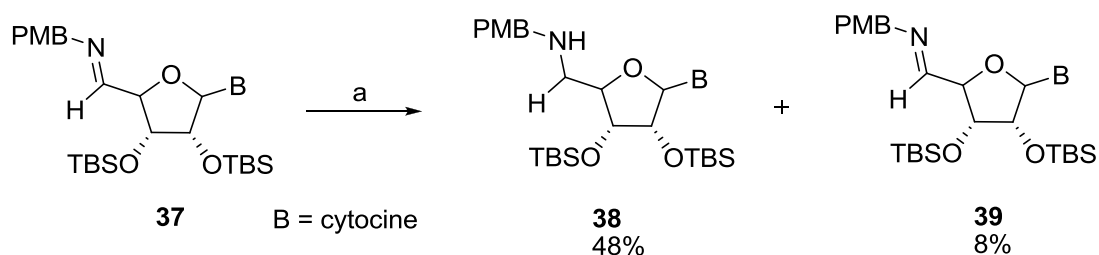
phosphite in the presence of a catalytic amount of indium(III) chloride (10 mol %) in dry THF at reflux or under sonication was achieved by Ranu *et. al.*<sup>10a</sup> with a better diastereoisomeric ratio 83:17 (Scheme 11).



**Scheme 11.** Reagents and conditions: (a)  $InCl_3$ , THF, reflux or sonication, 90-97%; dr up to 83:17.

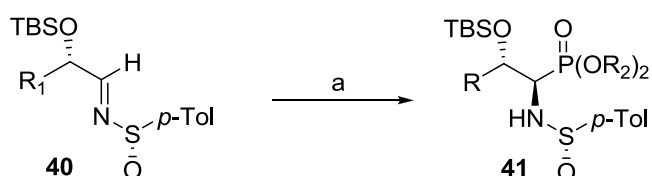
(b) Using chiral carbonyl compounds

Similarly, various carbonyl compounds have also been used for the asymmetric synthesis of  $\alpha$ -aminophosphonates. The aldehydes used for the asymmetric synthesis of  $\alpha$ -aminophosphonates includes *O*-protected  $\alpha$ -hydroxy aldehydes, carbohydrate based aldehydes, aldehydes derived from amino acids etc.<sup>19</sup> Generally asymmetric synthesis of  $\alpha$ -aminophosphonates has been achieved by employing both chiral amine as well as aldehyde *e.g* Chen *et al.*<sup>21</sup> studied the reaction of imine **37** prepared by the condensation of protected cytidine with *p*-methoxybenzylamine followed by addition of lithium diethyl phosphite afforded the  $\alpha$ -aminophosphonates **38** and **39** in good diastereomeric ratio (6:1) (Scheme 12).



**Scheme 12.** Reagents and conditions: (a) Diethylphosphite; LHMDS, THF, 0°C to rt.

In another example, synthesis of  $\alpha$ -aminophosphonates ( $S_S,IR,2S$ )-**41** was reported (Scheme 13) by Davis *et al.*<sup>22</sup> which involved addition of potassium salt of dialkylphosphite to the enantiopure *O*-protected  $\alpha$ -hydroxy sulfinimine ( $S_S,2S$ )-**40**. The product was obtained in good yield and excellent de (94%).

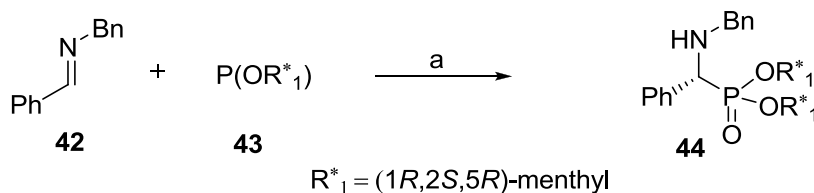


**Scheme 13.** Reagents and conditions: (a) Potassium salt of alkylphosphite, THF, -78 °C, 68-76%; de 94%.

(c) Using chiral phosphites

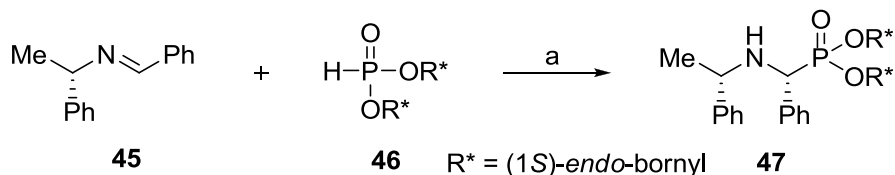
Chiral phosphites have also been employed for the asymmetric synthesis of  $\alpha$ -aminophosphonates. *Tris*[(1*R*,2*S*,5*R*)-menthyl] phosphite, chiral di-[(1*S*)-endo-bornyl] phosphite, chiral spirophosphoranes and phosphites obtained from chiral diol etc. were reported to affect the asymmetric synthesis of  $\alpha$ -aminophosphonates either independently or in conjunction with chiral amine or chiral aldehydes.<sup>19</sup>

Kolodiazhnyi *et al*<sup>23</sup> had reported the hydrophosphinylation reaction of imine **42** with *tris*[(1*R*,2*S*,5*R*)-menthyl] phosphite **43** under the catalytic influence of TMSCl to furnish  $\alpha$ -aminophosphonates **44** in good yield with moderate diastereoselectivity (de 50%).



**Scheme 14.** Reagents and conditions: (a) TMSCl, rt, de 50%.

Similarly, Kachkovskii *et al*<sup>24</sup> studied the addition of chiral di-[(1*S*)-endo-bornyl] phosphite **46** to (*S*)-**45** to afford  $\alpha$ -aminophosphonate (*R,S*)-**47** in 60% yield with 86% de (Scheme 15).



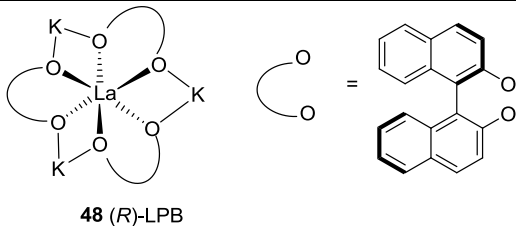
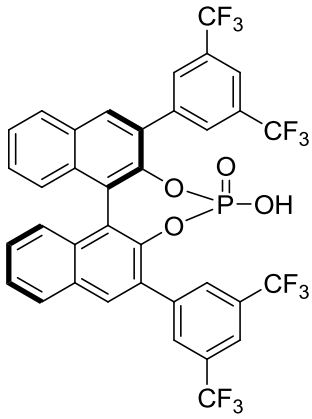
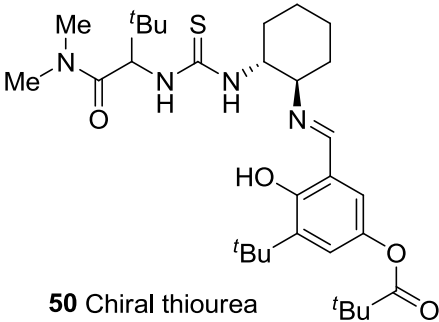
**Scheme 15.** Reagents and condition: (a) 80 °C, 60%; de 86%.

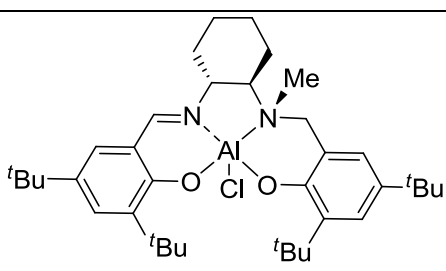
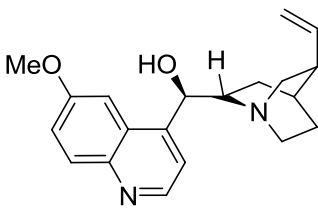


## (ii) Catalytic Asymmetric Synthesis

Catalytic enantioselective synthesis is the most important method for the asymmetric synthesis of  $\alpha$ -aminophosphonates.<sup>19,25</sup> Various catalytic systems have been developed for the asymmetric synthesis of  $\alpha$ -aminophosphonates such as lanthanoid/potassium/BINOL complex,<sup>25a</sup> cyclic phosphoric acid,<sup>25b</sup> chiral urea,<sup>25c</sup> complex (*R*)-Al(salalen)<sup>25d-e</sup> and quinine.<sup>25f</sup> The structure and scope of the catalysts are compiled in the Table 1.

**Table 1.** Catalytic enantioselective synthesis of  $\alpha$ -aminophosphonates.

Entry	Catalyst	Scope	Yield	Maximum de
1	 48 ( <i>R</i> )-LPB	Aliphatic aldehyde, aromatic amine, dialkylphosphite	25-86%	98%
2	 49 Chiral phosphoric acid	Aromatic/aliphatic aldehyde, aromatic amine, dialkylphosphite	72-97%	90%
3	 50 Chiral thiourea	Aromatic/aliphatic aldehyde, aromatic amine, dialkylphosphite	52-93%	99%

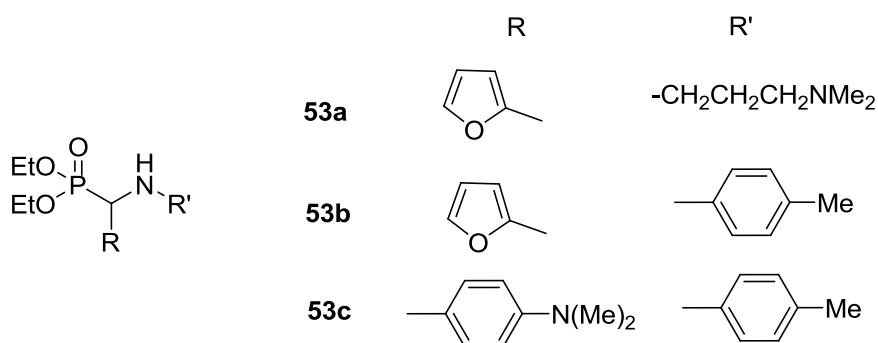
Entry	Catalyst	Scope	Yield	Maximum de
4	 <b>51 (R)-Al(Salalen)</b>	Aromatic aldehyde, aromatic amine, dialkylphosphite	92-99%	95%
5	 <b>52 Quinine</b>	Aromatic aldehyde, Boc-amine	50-83%	94%

### Biological Activities of $\alpha$ -Aminophosphonates

Troev *et al* had synthesized novel  $\alpha$ -aminophosphonates, **53a-c** and studied their antiproliferative effects against 4 human leukemic cell lines.<sup>26</sup> Among the tested compounds, aminophosphonate **53b** was the most potent cytotoxic agent. Except aminophosphonate **53b**, all the studied compounds **53a-c** were less active as compared to the reference anticancer drug, cisplatin.

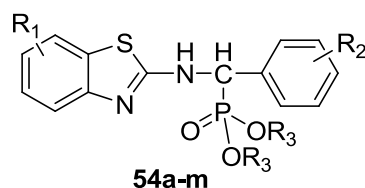
**Table 2.** Comparative cytotoxic activity of compounds **53a-c**

Cell line	IC <sub>50</sub> value ( $\mu$ M)			
	<b>53a</b>	<b>53b</b>	<b>53c</b>	Cisplatin
LAMA-84	> 400.0	71.2±2.4	119.4±6.3	18.2±1.7
K-562	352.9±11.7	22.9±0.9	42.4±3.0	25.7±2.1
HL-60	163.4±5.3	74.8±2.7	> 400.0	7.8±1.1
HL-60/Dox	190.0±4.7	115.2±7.1	107.2±4.1	14.5 ±1.4



Song *et al* had synthesized  $\alpha$ -aminophosphonates **54a-m** (Table 3) containing benzothiazole and fluorine moiety. The anticancer activities against PC3, A431, A375, and Bcap37 cells *in vitro* by the MTT method (Table 4) was evaluated of all the synthesized compounds **54a-m**.<sup>27</sup> The data revealed that compound **54c** is highly effective against PC3 cells and showed moderate activity against A431 cells.

**Table 3.** Synthesis of  $\alpha$ -aminophosphonates containing benzothiazole and fluorine moiety



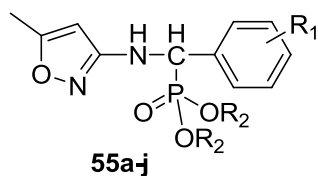
Entry	Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
1	<b>54a</b>	4-CH <sub>3</sub>	2-F	Et
2	<b>54b</b>	4-CH <sub>3</sub>	2-F	<sup>n</sup> Pr
3	<b>54c</b>	4-CH <sub>3</sub>	2-F	<sup>n</sup> Bu
4	<b>54d</b>	4-CH <sub>3</sub>	4-CF <sub>3</sub>	Me
5	<b>54e</b>	4-CH <sub>3</sub>	4-CF <sub>3</sub>	Et
6	<b>54f</b>	4-CH <sub>3</sub>	4-CF <sub>3</sub>	<sup>i</sup> Pr
7	<b>54g</b>	4-CH <sub>3</sub>	4-CF <sub>3</sub>	<sup>n</sup> Bu
8	<b>54h</b>	6-OCH <sub>3</sub>	2-F	Me
9	<b>54i</b>	6-OCH <sub>3</sub>	2-F	Et
10	<b>54j</b>	6-OCH <sub>3</sub>	2-F	<sup>n</sup> Pr
11	<b>54k</b>	6-OCH <sub>3</sub>	2-F	<sup>i</sup> Pr
12	<b>54l</b>	6-OCH <sub>3</sub>	2-F	<sup>n</sup> Bu
13	<b>54m</b>	6-OCH <sub>3</sub>	4-F	Et

**Table 4.** Anticancer activities of  $\alpha$ -aminophosphonates **54a-m** containing benzothiazole and fluorine moiety

Compound	PC3 cells			A431 cells			A375 cells			Bcap 37 cells		
	1 $\mu$ M	5 $\mu$ M	10 $\mu$ M	1 $\mu$ M	5 $\mu$ M	10 $\mu$ M	1 $\mu$ M	5 $\mu$ M	10 $\mu$ M	1 $\mu$ M	5 $\mu$ M	10 $\mu$ M
<b>54a</b>	3.51	22.9	41.9	17.7	23.3	35.3	-13.9	-6.1	9.2	35.8	42.6	58.9
<b>54b</b>	21.7	29.4	43.3	15.9	27.3	31.7	-0.2	4.8	4.3	-18.9	0.8	21.7
<b>54c</b>	52.1	86.0	89.1	32.1	49.0	72.1	11.2	25.6	32.1	10.0	29.0	38.1
<b>54d</b>	4.0	4.6	8.9	22.1	26.0	47.3	1.8	19.2	20.8	-13.2	-0.8	2.5
<b>54e</b>	12.6	13.5	49.3	0.36	2.06	16.9	-10.2	1.6	14.2	-5.2	9.8	16.7
<b>54f</b>	6.3	15.3	25.9	17.3	20.5	29.5	-1.3	8.6	9.1	-0.7	11.2	22.7
<b>54g</b>	5.0	31.6	36.0	37.7	40.8	57.4	-3.2	21.6	31.5	0.1	9.2	20.3
<b>54h</b>	21.4	26.6	33.3	1.9	7.9	8.64	4.3	10.1	11.4	11.9	17.8	25.1
<b>54i</b>	16.8	22.5	38.9	4.5	12.3	22.1	7.5	7.9	15.7	20.5	34.5	49.4
<b>54j</b>	5.0	15.8	28.7	35.3	49.2	58.1	10.3	13.9	23.8	21.3	25.1	27.1
<b>54k</b>	-8.5	2.1	18.5	-7.4	7.5	16.7	-4.3	1.7	2.8	39.7	43.2	50.4
<b>54l</b>	-6.6	5.3	13.2	-3.7	5.4	28.8	-6.2	3.5	17.4	23.6	38.2	44.2
<b>54m</b>	32.1	41.0	49.5	11.2	20.9	31.2	8.0	10.9	34.5	10.0	39.0	49.9

$\alpha$ -Aminophosphonates **55a-j** having isoxazole and fluorine moiety were synthesized using one step protocol under the influence of ultrasound irradiation by Song *et al.*<sup>28</sup> These compounds (**55a-j**) were evaluated for their antitumor activities against PC3 and A431 cells and were found to possess moderate antitumor activities (Table 5).

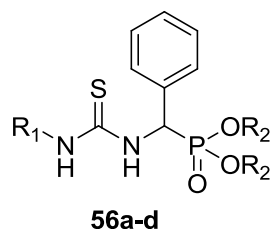
**Table 5.** Inhibition rate of compounds 55a-j against PC3 and A431 cell proliferation



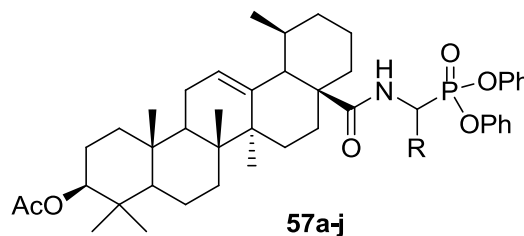
Entry	Compound	R <sub>1</sub>	R <sub>2</sub>	PC3 cells	A431 cells
1	<b>55a</b>	2-F	Me	22.1	10.9
2	<b>55b</b>	2-F	Et	40.7	29.8
3	<b>55c</b>	2-F	<sup>n</sup> Pr	47.8	34.5
4	<b>55d</b>	2-F	<sup>i</sup> Pr	58.9	48.0
5	<b>55e</b>	2-F	<sup>n</sup> Bu	65.1	55.3
6	<b>55f</b>	4-F	Me	50.2	27.9
7	<b>55g</b>	4-F	Et	60.0	34.5
8	<b>55h</b>	4-F	<sup>n</sup> Pr	70.0	65.8
9	<b>55i</b>	4-F	<sup>i</sup> Pr	78.3	69.0
10	<b>55j</b>	4-F	<sup>n</sup> Bu	66.8	67.3

Song *et al.*<sup>29</sup> had synthesized and studied antiviral activities of chiral thiourea derivatives bearing  $\alpha$ -aminophosphonate moiety. It was observed that compounds **56g**, **56e**, **56k**, and **56m** showed the same curative effects of TMV (inhibitory rate 54.8, 50.5, 50.4, and 50.4%, respectively) as the commercial product, ningnanmycin (56.2%).

Deng *et al.*<sup>30</sup> had synthesized  $\alpha$ -aminophosphonate derivatives of ursolic acid (UA). It was found that the derivatives of UA exhibit specific anti-HIV activity. They inhibit gp120-CD4 interaction and thereby they are considered to be an HIV-1 entry inhibitor (Table 7).

**Table 6.** *In vivo* antiviral activities of compounds **56a-d**

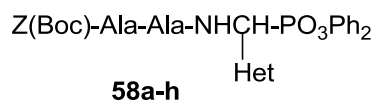
Entry	Compounds	R <sub>1</sub>	R <sub>2</sub>	TMV curative effect (%)			EC <sub>50</sub> (μg/mL)
				500 μg/mL	250 μg/mL	125 μg/mL	
1	<b>56a</b>	<sup>n</sup> Pr	( <i>S</i> )-1-phenylethyl	50.5	37.7	30.9	399.9
2	<b>56b</b>	<sup>n</sup> Pr	( <i>S</i> )-1-cyclohexylethyl	54.8	49.0	39.1	239.8
3	<b>56c</b>	<sup>i</sup> Pr	( <i>S</i> )-1-cyclohexylethyl	50.4	36.7	29.0	413.4
4	<b>56d</b>	<sup>n</sup> Bu	( <i>S</i> )-1-cyclohexylethyl	50.4	37.2	32.9	378.9
5	Ningnamycin			56.2	50.7	41.9	227.0

**Table 7.** HIV entry inhibitor activity of compound **57a-j**

Compound	<b>57a</b>	<b>57b</b>	<b>57c</b>	<b>57d</b>	<b>57e</b>	<b>57f</b>	<b>57g</b>	<b>57h</b>	<b>57i</b>	<b>57j</b>
R	H	<sup>n</sup> Pr	<i>p</i> -OMePh	<sup>n</sup> Bu	Ph	<i>p</i> -MePh	<i>p</i> -ClPh	<i>o</i> -ClPh	2,4-Cl <sub>2</sub> Ph	<i>o</i> -NO <sub>2</sub> Ph
Inhibition (%)	18	17	20	0	11	6	0	5	12	2

Boduszek *et al*<sup>31</sup> had synthesized novel heterocyclic peptidyl phosphonates **58a-h**. The synthesized compounds **58a-h** were tested for enzyme inhibition and proved to be irreversible inhibitors of chymotrypsin (ChT), human neutrophil elastase (HNE) and porcine pancreatic elastase (PPE) (Table 8).

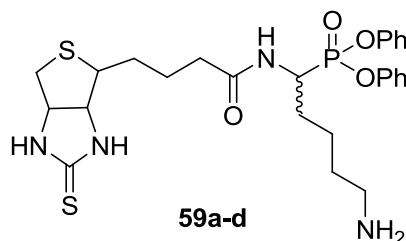
**Table 8.** Rate constants of inhibition of chymotrypsin, PPE and HNE by phosphono-peptidase



Entry	Compound	Z/Boc	Het	ChT	PPE	HNE
1	<b>58a</b>	Z	Furyl	28	159	370
2	<b>58b</b>	Boc	Furyl	NI	600	135
3	<b>58c</b>	Z	Thienyl	152	253	140
4	<b>58d</b>	Boc	Thienyl	17	856	41
5	<b>58e</b>	Z	Thienyl	26	25	13
6	<b>58f</b>	Boc	Thienyl	5	124	13
7	<b>58g</b>	Z	Pirydyl	8	NI	NI
8	<b>58h</b>	Z	Pirydyl	26	4	NI

Howthorne *et al*<sup>32</sup> reported synthesis of biotinylated diphenyl phosphonates **59a-d** and have successfully shown that diphenyl phosphonate **59a-d** are specific inhibitors of serine proteases.

**Table 9.** Kinetic constants for the inactivation of trypsin and plasmin by diphenyl phosphonate analogues **59a-d**



Entry	Compound	Trypsin ( $K_i$ $\mu\text{M}$ )	Plasmin ( $K_i$ $\mu\text{M}$ )
1	Cbz-Lys(OPh) <sub>2</sub> ( <b>59a</b> )	12.1	ND
2	Bio-Lys(OPh) <sub>2</sub> ( <b>59b</b> )	12.8	28.6
3	Cbz-Orn(OPh) <sub>2</sub> ( <b>59c</b> )	14.1	ND
4	Bio-Orn(OPh) <sub>2</sub> ( <b>59d</b> )	15.6	2.0

Power *et al*<sup>33</sup> reported synthesis of peptidyl derivatives of diphenyl ( $\alpha$ -aminoalkyl)phosphonates **60-72**. The compounds **60-72** were tested for enzyme inhibition and were found to be effective inhibitors of serine proteases at low concentration.

**Table 10.** Rate constants for inactivation of serine protease by peptides with C-terminal diphenyl ( $\alpha$ -aminoalkyl)phosphonates **60-72**

Entry	Compound	Chymotrypsin $K_i$ ( $\mu\text{M}$ )	PPE $K_i$ ( $\mu\text{M}$ )	HLE $K_i$ ( $\mu\text{M}$ )
1	Z-Pro-Val <sup>P</sup> (OPh) <sub>2</sub> ( <b>60</b> )	8.2	10	5.3
2	Z-Pro-Phe <sup>P</sup> (OPh) <sub>2</sub> ( <b>61</b> )	8.2	8.2	4.3
3	Z-Ala-Ala-Val <sup>P</sup> (OPh) <sub>2</sub> ( <b>62</b> )	8.2	24.3	13.5
4	Meo-Suc-Ala-Ala-Pro-Nva <sup>P</sup> (OPh) <sub>2</sub> ( <b>63</b> )	8.2	8.6	95
5	Meo-Suc-Ala-Ala-Ala-Val <sup>P</sup> (OPh) <sub>2</sub> ( <b>64</b> )	90.1	9.1	5
6	Meo-Suc-Ala-Ala-Ala-Phe <sup>P</sup> (OPh) <sub>2</sub> ( <b>65</b> )	5.3	105	58
7	Meo-Suc-Ala-Ala-Pro-Val <sup>P</sup> (OPh) <sub>2</sub> ( <b>66</b> )	180	9	4.9
8	Meo-Suc-Ala-Ala-Pro-Leu <sup>P</sup> (OPh) <sub>2</sub> ( <b>67</b> )	26	26	14.4
9	Meo-Suc-Ala-Ala-Pro-Phe <sup>P</sup> (OPh) <sub>2</sub> ( <b>68</b> )	11	100	50
10	Meo-Suc-Ala-Ala-Pro-Met <sup>P</sup> (OPh) <sub>2</sub> ( <b>69</b> )	30	30	76
11	Boc-Val-Pro-Val <sup>P</sup> (OPh) <sub>2</sub> ( <b>70</b> )	8.2	8.7	4.5
12	Z-Phe-Pro-Phe <sup>P</sup> (OPh) <sub>2</sub> ( <b>71</b> )	4.6	92	8.2
13	Suc-Val-Pro-Phe <sup>P</sup> (OPh) <sub>2</sub> ( <b>72</b> )	5.5	8.2	4.1

## (ii) $\beta$ -Aminophosphonates

$\beta$ -Aminophosphonates are the isosteres of  $\beta$ -amino acids and therefore, they occupy an important place in chemistry as well as in biology revealing diverse and interesting biological properties.<sup>34</sup>  $\beta$ -Aminophosphonates are also found in nature as a consequence, 2-aminoethylphosphonic acid from *Ciliate protozoa* was isolated in 1959 by Horiguchi and Kandatsu. Later on various  $\beta$ -aminophosphonic acids and esters have been isolated from various living organisms.<sup>35</sup>

### Synthesis of $\beta$ -Aminophosphonates

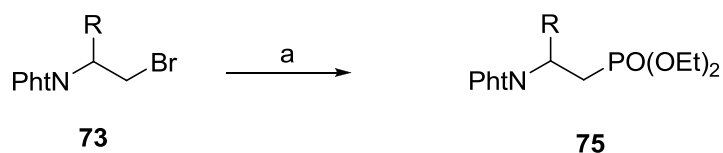
Depending on the type of bond formed in the reaction, synthesis of  $\beta$ -aminophosphonates could be grouped under three categories *viz* (i) C-P, (ii) C-C and (iii) C-N bond formation.<sup>34</sup>

### (i) C-P bond formation

C-P bond formation is one of the most often used reactions for the synthesis of  $\beta$ -aminophosphonates.<sup>34</sup> This includes (a) Arbuzov reaction of phosphites with alkyl halides, (b) substitution reaction of  $\beta$ -amino halides or  $\beta$ -amino tosylates, (c) nucleophilic substitution of phosphites to aziridines, (d) hydrophosphinylation of  $\alpha$ -amino aldehydes and (e) addition of phosphites to unsaturated C-C bond.

#### (a) Arbuzov reaction

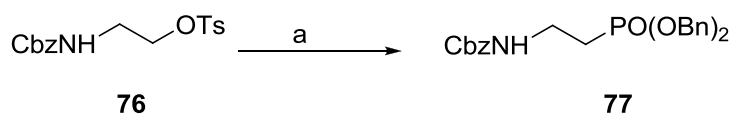
Arbuzov reaction is the simple route to the synthesis of  $\beta$ -aminophosphonates. When *N*-(bromoethyl)phthalimides **73** was reacted with triethyl phosphite under reflux conditions, phthaloylaminophosphonate **75** was obtained (Scheme 15).<sup>36</sup>



**Scheme 16.** Reagents and conditions: (a) P(OEt)<sub>3</sub> (**74**), reflux, 12 h, 30-95%.

#### (b) Substitution reaction of $\beta$ -amino halides or $\beta$ -amino tosylates

Alkylation of phosphonates can also be performed with  $\beta$ -amino alkyl tosylates. Tosylate **76** on treatment with sodium dibenzyl phosphinate furnished Cbz-*N*-protected dibenzyl (2-aminoethyl) phosphonate **77** in 78% yield (Scheme 16).<sup>37</sup>



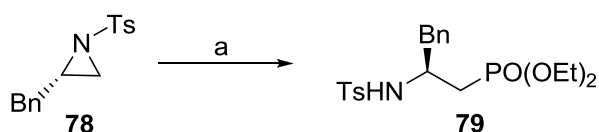
**Scheme 17.** Reagents and conditions: (a) (BnO)<sub>2</sub>PONa, 78%.

#### (c) Nucleophilic substitution of phosphites to aziridines

Ring opening of three membered strained nitrogen heterocycles can also be used for the preparation of  $\beta$ -aminophosphonates.<sup>34</sup> *N*-Tosylaminophosphonic derivatives **79** were synthesized in good yields by nucleophilic ring opening of



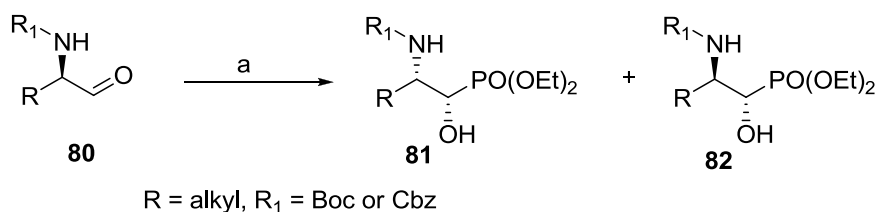
aziridine **78** with excess of diethyl phosphonates in THF at room temperature, (Scheme 18).<sup>38</sup>



**Scheme 18.** Reagents and conditions: (a)  $(\text{EtO})_2\text{PONa}$ , 90%.

(d) Hydrophosphinylation of  $\alpha$ -amino aldehydes

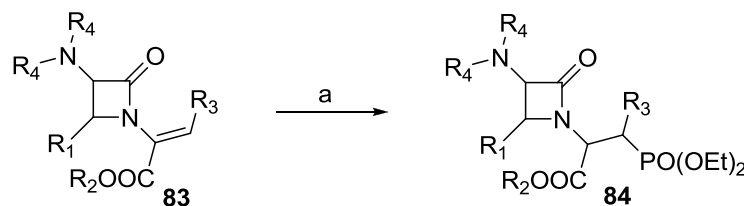
Hydrophosphinylation of amino-functionalized aldehydes or ketones is generally carried out by base or by Lewis-acid affording  $\beta$ -amino- $\alpha$ -hydroxy phosphonates.<sup>34</sup> When *N*-protected- $\alpha$ -aminoaldehydes **80** was subjected to hydrophosphinylation, the corresponding  $\beta$ -amino- $\alpha$ -hydroxyphosphonates **81** and **82** were obtained with high diastereoselectivity (Scheme 19).<sup>39</sup> The stereoselectivity of this reaction was dependent on the choice of base *e.g.* with DBU as a base equimolecular mixture of *syn*- and *anti*-diastereomers were obtained however, with fluoride as base, reaction favoured stereoselection for the formation of *syn* product **81**.



**Scheme 19.** Reagents and conditions: (a)  $(\text{EtO})_2\text{PONa}$ , 42-95%.

(e) Addition of phosphites to unsaturated C-C

Michael addition of various phosphorus reagents to Michael acceptors (Scheme 20) is also an effective way to synthesize  $\beta$ -aminophosphonates.<sup>34</sup> When acrylic acid derivative **83** was reacted with diethyl phosphite using a catalytic amount of NaH, adduct **84** was obtained in good yield.<sup>40</sup>



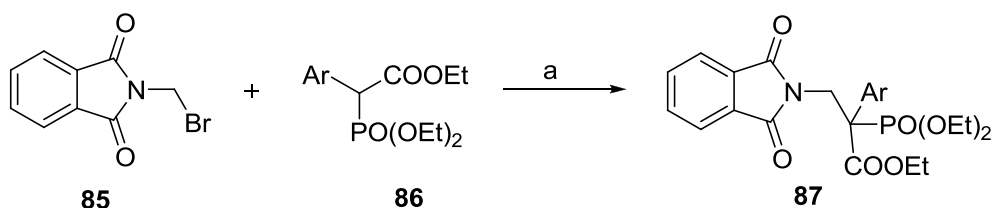
**Scheme 20.** Reagents and conditions: (a) (EtO)<sub>2</sub>POH, NaH, 20-99%.

## (ii) C-C bond formation

$\beta$ -Aminophosphonates have been synthesized by using C-C bond formation reaction which includes (a) addition of carbanion to  $\alpha$ -haloaminoderivative, (b) addition of carbanion to imines and iminium salts and (c) cycloaddition reactions.<sup>34</sup>

### (a) Addition of carbanion to $\alpha$ -haloaminoderivative

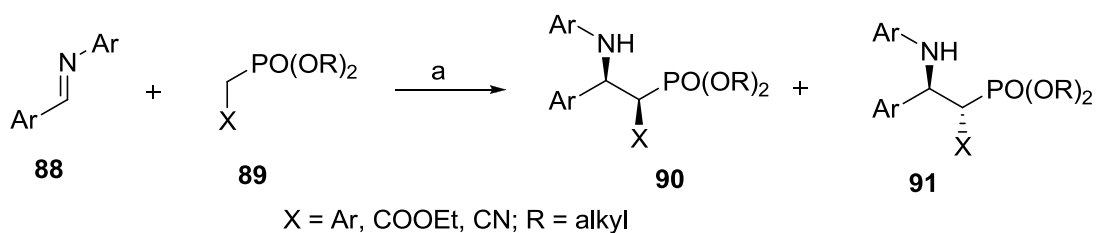
It is one of the general methods for the synthesis of  $\beta$ -aminophosphonates. Initially, carbanions derived from phosphonoacetate **86** was generated using base like NaH followed by *in situ* substitution reaction with amino bromide **85** resulted in formation of  $\beta$ -aminophosphonate **87**, which is precursor of potential GABA<sub>B</sub> receptor antagonists.<sup>41</sup>



**Scheme 21.** Reagents and conditions: (a) NaH, THF, 63%.

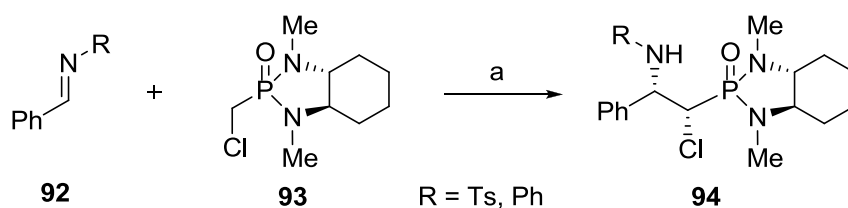
### (b) Addition of carbanion to imines and iminium salts

This is one of the most frequently used methods for the synthesis of  $\beta$ -aminophosphonates and deals with the addition of carbanions derived from phosphonates to imines or sulfinimines.<sup>34</sup> Kirilov *et al*<sup>42</sup> reported synthesis of  $\beta$ -aminophosphonates, *syn*-**90** and *anti*- $\alpha$ -aryl- $\beta$ -aminophosphonates **91** which involved generation of carbanions from phosphonates **89** using 0.5 equiv of NaNH<sub>2</sub> and *in situ* addition to Schiff's bases **88** in ether or liquid ammonia (Scheme 22).



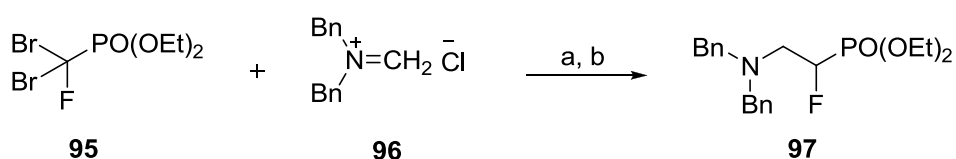
**Scheme 22.** Reagents and conditions: (a) base.

Hanessian *et al*<sup>43</sup> studied the stereoselective addition of carbanions derived from optically pure  $\alpha$ -chloromethyl bicyclic phosphonamide **93** to imines **92** to afford  $\beta$ -amino- $\alpha$ -chlorophosphonamides **94** (Scheme 23).



**Scheme 23.** Reagents and conditions: (a) *n*BuLi, -78 °C, THF.

Iminium salts can also be used for the preparation of  $\beta$ -aminophosphonates as it also contains a C-N double bond.<sup>34</sup> O'Hagan *et al*<sup>44</sup> reported the addition of carbanions derived from diethyl (dibromofluoromethyl)phosphonate **95** to iminium salt **96**. The carbanion was formed by double halogen exchange with *n*-BuLi in the presence of chlorotrimethylsilane (TMSCl) and was then added to iminium chloride **96** followed by desilylation afforded adduct **97** (Scheme 24).

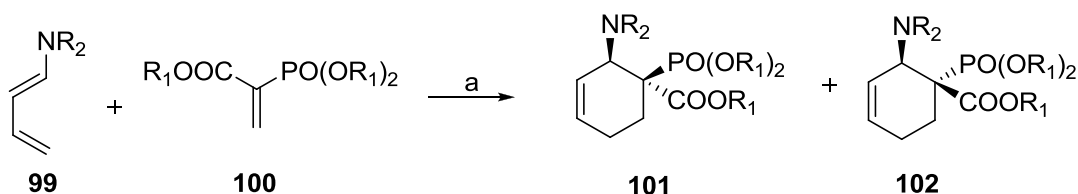


**Scheme 24.** Reagents and conditions: (a) *n*BuLi, TMSCl, -78 °C, THF; (b) LiOEt, EtOH, 0°C, aq. NH<sub>4</sub>Cl.

### (c) Cycloaddition reactions

Recently, preparation of  $\beta$ -aminophosphonates has been achieved by [4+2] cycloaddition strategy using aminodienes and phosphono-dienophiles (Scheme 23)<sup>45</sup> through carbon-carbon bond formation. The synthesis of  $\beta$ -aminophosphonates **101**

and **102** was accomplished by [4+2] cycloaddition of aminodienes **99** to vinylphosphonates dienophiles **100**.



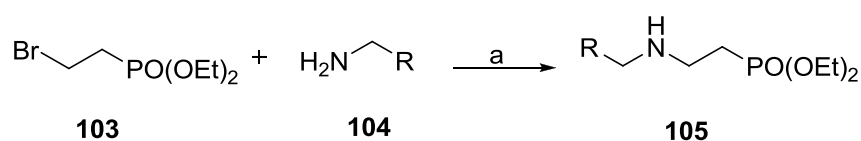
**Scheme 25.** Reagents and conditions: (a) Hydroquinone, MeCN, 65 °C, 65-93%, dr 80:20.

### (iii) C-N bond formation

β-Aminophosphonates have been synthesized by using C-N bond formation reactions which includes (a) halide displacement with amines, (b) Michael addition of amine to vinylphosphonates, (c) aminodihydroxylation, (d) ammonolysis of epoxyphosphonates, (e) addition of azide to cyclic sulphites and (f) Hoffmann reaction.<sup>34</sup>

#### (a) Halide displacement with amines

β-Aminoalkylphosphonates is synthesized by the halide displacement reaction of haloalkylphosphonates with amine.<sup>34</sup> The simplest example is shown in Scheme 24 where Gao *et al*<sup>46</sup> had synthesized β-aminoalkylphosphonates **105** using bromide displacement reaction in compound **103** by amine **104** in presence of base like NaH.

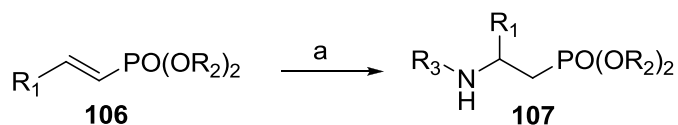


**Scheme 26.** Reagents and conditions: (a) base.

#### (b) Michael addition of amine to vinylphosphonates

Michael reaction is the conjugate addition of nucleophiles to α,β-unsaturated compounds which is considered to be one of the most important and particularly useful methods for carbon-carbon and carbon-heteroatom bond formation. β-Aminophosphonates through Michael addition reaction have been well documented in the literature.<sup>34</sup>

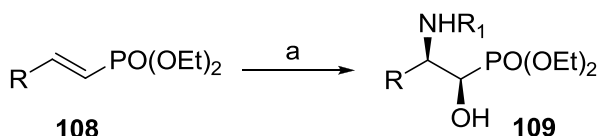
Floch *et al.*<sup>47</sup> reported the preparation of **107** by using Michael addition reaction of amine to the vinylphosphonates **106** (Scheme 27).



**Scheme 27.** Reagents and conditions: (a)  $R_3NH_2$ , EtOH.

### (c) Aminodihydroxylation

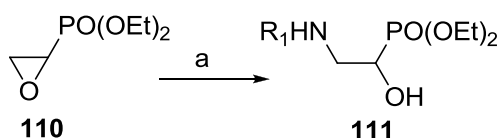
Aminohydroxylation of the vinylphosphonates is also one of the useful methods for the synthesis of  $\beta$ -aminophosphonates. Vinylphosphonates **108** was subjected to oxyamination using Os-(VIII) and the cinchona alkaloid ligand (DHQD)<sub>2</sub>-PHAL and chloramine-T hydrate in <sup>t</sup>BuOH-H<sub>2</sub>O (1:1, v/v) at room temperature (Scheme 28) afforded the  $\beta$ -amino- $\alpha$ -hydroxy derivatives **109**.<sup>48</sup> The reaction was completed in 2-24 h to reach >95% conversion.



**Scheme 28.** Reagents and conditions: (a) Admix- $\alpha$ , <sup>t</sup>BuOH-H<sub>2</sub>O, Na<sub>2</sub>SO<sub>3</sub>, 15-92%.

### (d) Ammonolysis of epoxyphosphonates

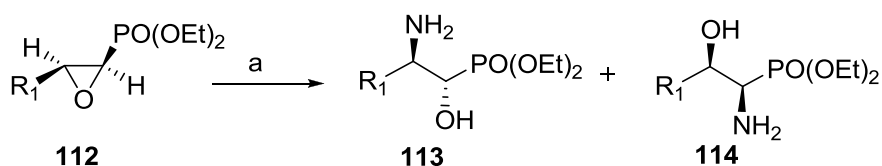
$\beta$ -Aminophosphonate **111** was synthesized by the addition of nitrogen nucleophiles like ammonia or aniline to epoxide **110** (Scheme 29).<sup>49</sup>



**Scheme 29.** Reagents and conditions: (a)  $R_1NH_2$ , 90%.

Similarly, when the *trans*-oxirane **112** was reacted with excess of ammonia in MeOH furnished only one diastereoisomer of the desired product **113** in high

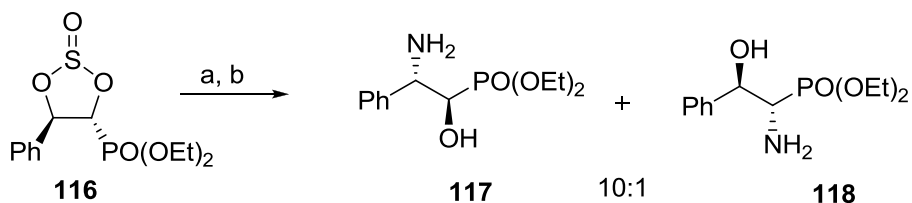
regioselectivity (Scheme 30).<sup>50</sup> Only in case of  $R_1 = o\text{-MeO-C}_6\text{H}_4$  the *syn*-diastereoisomer **114** was obtained in a ratio of 90:10.



**Scheme 30.** Reagents and conditions: (a) aq.  $\text{NH}_3$ , MeOH, rt, 42-59%.

(e) Addition of azide to cyclic sulphites

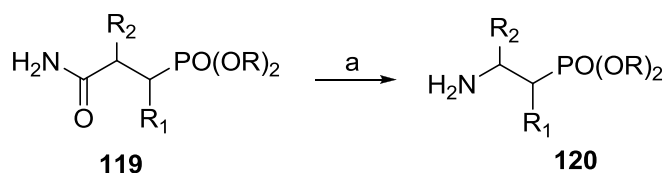
Cyclic sulphites can be opened with *N*-nucleophiles to furnish  $\beta$ -aminophosphonates *e.g.* ring opening of cyclic sulfites **116** with  $\text{NaN}_3$  yielded azidoxyphosphonates, which were subsequently reduced with  $\text{H}_2/\text{Pd-C}$  to furnish optically pure hydroxyaminophosphonates **117** and **118** in almost quantitative yield (Scheme 31).<sup>51</sup>



**Scheme 31.** Reagents and conditions: (a)  $\text{NaN}_3$ , DMF; (b)  $\text{H}_2/\text{Pd-C}$ , 80%.

(f) Hoffmann reaction

Finkelstein was the first to describe synthesis of  $\beta$ -aminoethylphosphonates **120** by following Hofmann rearrangement of amides **119** (Scheme 30).<sup>52</sup>



**Scheme 32.** Reagents and conditions: (a)  $\text{Br}_2$ , NaOH.

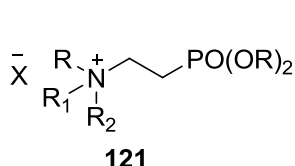
## Biological Activities of $\beta$ -Aminophosphonates

Clement *et al* has shown that  $\beta$ -aminophosphonates can be used as non-viral mediated gene transfer agents.<sup>53</sup> The synthetic vectors which are able to complex DNA carry the resulting lipoplex through cell membranes and then deliver the DNA in (or close to) the nucleus in order to replace a deficient gene. The search of such synthetic vector is of great importance. The basic nature of biological amines and the fact that the cationic or polycationic charge is always carried by nitrogen atoms in these lipids. This prompted Clement *et al*<sup>53</sup> to synthesize and study quaternary ammonium compounds **121** as gene transfer agents.

Hakimelahi *et al* have synthesized  $\beta$ -aminophosphonate containing  $\beta$ -lactam moiety **122** and studied their carbapenem antibiotic activity.<sup>54</sup>

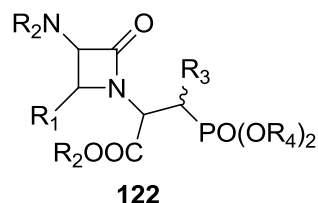
Guillerm *et al* have designed and synthesized  $\beta$ -aminophosphonate and carried out antifungal evaluation as a bisubstrate analogue inhibitor **123** for glycosyltransferases.<sup>55</sup>

Wester *et al.* had designed and synthesized orally active norstatine renin inhibitors **124**.<sup>56</sup>



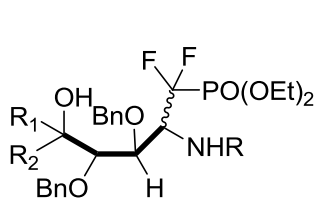
R, R<sub>1</sub>, R<sub>2</sub> = C<sub>14</sub>H<sub>29</sub>, C<sub>18</sub>H<sub>35</sub>, C<sub>38</sub>H<sub>77</sub>  
X = halogen triflate

Non-viral vector mediated gene transfer

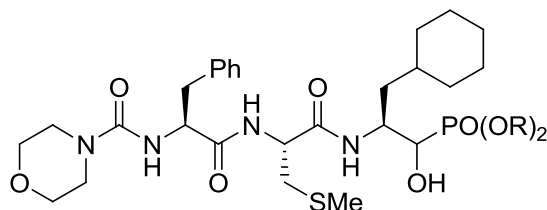


R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> = alkyl

Carbapenem antibiotic



Glycosyl transferase inhibitor



R = Me, Et, Bn

Renin inhibitor

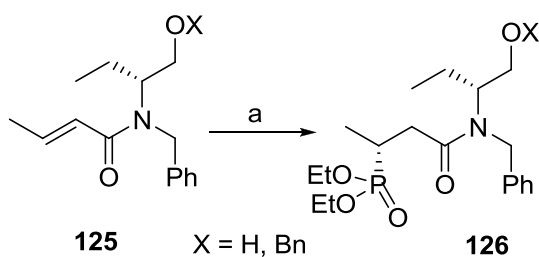
**Figure 2.** Biological activities of  $\beta$ -aminophosphonate.

### (iii) $\gamma$ -Aminophosphonates

The development of methods for the preparation of  $\gamma$ -aminophosphonate is important and currently attracting interest<sup>57-63</sup> of several research groups worldwide. A lot of general synthetic methods exist for the preparation of  $\alpha$ -aminophosphonates and  $\beta$ -aminophosphonates. However,  $\gamma$ -aminophosphonates have been scarcely described. Depending on the type of bond formed in the reaction, the syntheses of  $\gamma$ -aminophosphonates have been also classified as (i) C-P, (ii) C-C and (iii) C-N bond formation reactions.

#### (i) C-P bond formation

Quirion et al had reported synthesis of  $\gamma$ -aminophosphonates using C-P bond formation reaction.<sup>57</sup> The method is based on the Michael addition reaction of diethyl phosphite to various chiral  $\alpha,\beta$ -unsaturated carboxylic amides **125** derived from chiral amino alcohols. Initially diethyl phosphite was treated with base and then the resulting anion was reacted with **125** in THF at  $-78^\circ\text{C}$  resulted in the formation of Michael adduct **126** in moderate yield. The yields and the de depend on the base used. The best results were obtained with NaH as base (yield 50 % and de 92 %).<sup>57</sup>



**Scheme 33.** Reagents and conditions: (a) Diethylphosphite, Base.

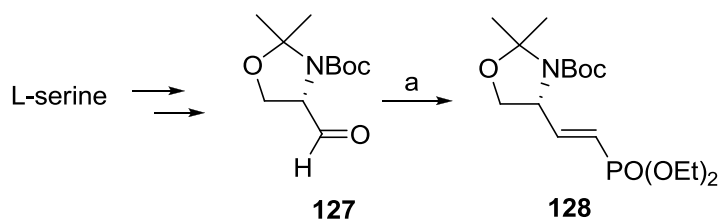
#### (ii) C-C bond formation

Synthesis of  $\gamma$ -aminophosphonates using C-C bond formation reaction includes (a) Wittig type reaction, (b) addition of carbanion derived from phosphonate to carbonyl compound and (c) metathesis reaction.



(a) Wittig type reaction

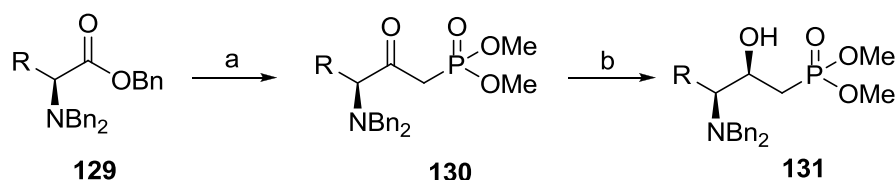
Macdonald *et al* had reported synthesis of  $\gamma$ -aminophosphonates using Horner–Wadsworth–Emmons olefination reaction of Garner’s aldehyde **127** with tetraethyl methylenebisphosphonate affording **128** in 75 % yield (Scheme 34).<sup>58</sup>



**Scheme 34.** Reagents and conditions: (a) Tetraethyl methylenebisphosphonate, <sup>t</sup>BuLi, THF, -78 °C-rt, overnight, 75%.

(b) Addition of carbanion derived from phosphonate to carbonyl compound

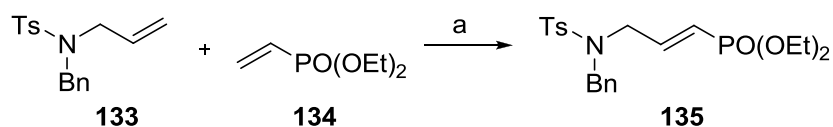
Ordonez *et al* carried out synthesis of  $\gamma$ -*N,N*-dibenzylamino- $\beta$ -ketophosphonates **130** from readily available (*S*)-tribenzylated amino acids **129** by reacting it with lithium dimethylmethylphosphonate followed by reduction with catecholborane at -20°C affording  $\gamma$ -amino- $\beta$ -hydroxyphosphonates **131** in high diastereoselectivity and good yield (50-89 %, de >98 %).<sup>59</sup>



**Scheme 35.** Reagents and conditions: (a) Lithium dimethyl methylphosphonate, -78°C, THF; (b) catechol borane, -20 °C, THF.

(c) Metathesis reaction

Fadel *et al* had synthesized *trans*- $\gamma$ -aminovinylphosphonate **135** by cross-metathesis of allyl amide **133** and vinylphosphonate **134** using Grubbs II generation catalyst (5 mol %) (Scheme 34).<sup>60</sup>



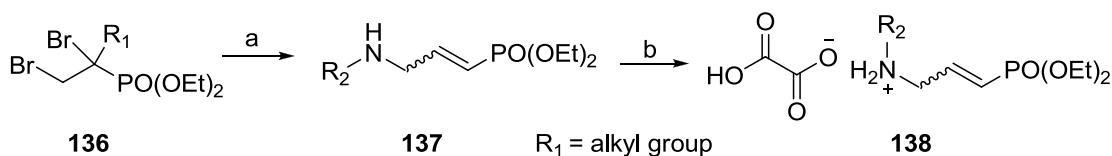
**Scheme 36.** Reagents and conditions: (a) Grubbs II, DCM, reflux, 20h, 61%.

### (iii) C-N bond formation

Synthesis of  $\gamma$ -aminophosphonates using C-N bond formation reaction includes (a) halide displacement reaction, (b) epoxide opening reaction and (c) Tsuji-Trost reaction.

#### (a) Halide displacement reaction

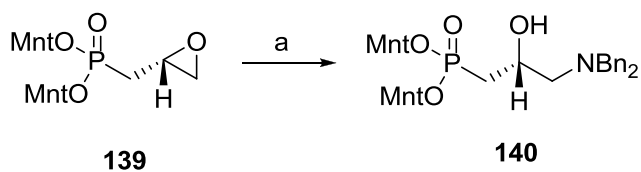
Stevens *et al*<sup>61</sup> had reported synthesis of  $\gamma$ -amino- $\alpha,\beta$ -unsaturated phosphonates *via* substitution-elimination sequence of dibromopropylphosphonates with primary amines. The procedure consists of the addition of one equivalent of the dibromophosphonate **136** to three equivalents of primary amine in dichloromethane or ether followed by precipitation of the salt using oxalic acid (Scheme 37) to form  $\gamma$ -aminophosphonates **138**.



**Scheme 37.** Reagents and conditions: (a)  $\text{R}_2\text{NH}_2$ ;  $\text{Et}_2\text{O}$ ; reflux; (b) Oxalic acid.

#### (b) Epoxide opening reaction

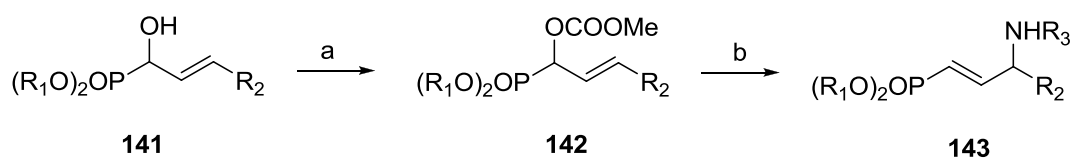
Kolodiaznyi *et al*<sup>62</sup> had synthesized  $\gamma$ -aminovinylphosphonate by epoxide ring opening of epoxyphosphonate. Epoxide ring in the phosphonate **139** was opened regioselectively with *N,N*-dibenzylamine at C-3 to yield the crystalline dimethyl (*R*)-2-hydroxy-3-(*N,N*-dibenzylamino)propylphosphonate **140** (Scheme 38).



**Scheme 38.** Reagents and conditions: (a)  $\text{Bn}_2\text{NH}$ , MeOH, reflux, 10 h, 70%, de >98%.

(c) Tsuji-Trost reaction

Spilling *et al*<sup>63</sup> had reported synthesis of  $\gamma$ -aminovinylphosphonate by employing palladium catalysed Tsuji-Trost reaction of allylic  $\alpha$ -hydroxyphosphonate (Scheme 39).



**Scheme 39.** Reagents and conditions: (a) Pyridine, Methylchloroformate, rt 12 h; (b)  $\text{Pd}(\text{PPh}_3)_4$ ,  $\text{R}_3\text{NH}_2$ , THF.

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## **Chapter 2**

**Synthesis of Aminophosphonate Derivatives of  
Hydroxyethylamine (HEA) Isostere and  
Their Anti-HIV Activity**

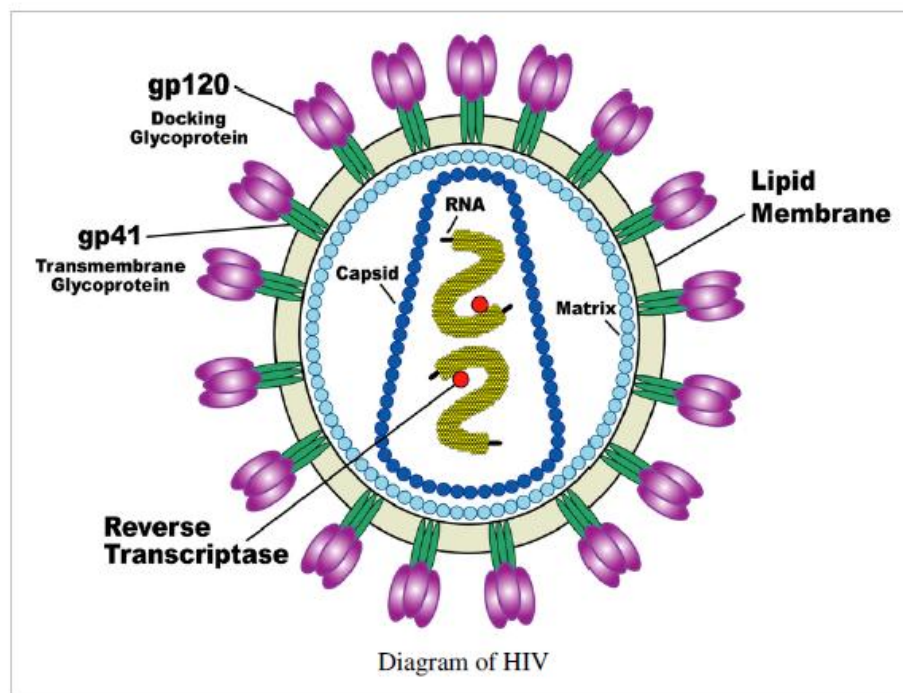
## Introduction

Acquired immune deficiency syndrome (AIDS) is a disease of the human immune system caused by the human immunodeficiency virus (HIV). AIDS was first observed in USA (1980-81) in previously healthy homosexual men and intravenous drug abusers with increased occurrence of *Pneumocystis carinii* pneumonia and Kaposi's cancer and opportunistic infections.<sup>1</sup> The causative agent of AIDS was identified by Robert Gallo and Luc Montagnier independently as a human retrovirus.<sup>2,3</sup> Initially, three different names, human T lymphotropic virus III (HTLVIII), lymphadenopathy-associated virus (LAV), and AIDS-associated retrovirus (ARV) were acronymed to the virus.<sup>3,4</sup> From 1986 onwards the virus was known as human immunodeficiency virus (HIV).<sup>5</sup> Later HIV-2, was isolated from patients in West Africa.<sup>6</sup> Both the HIV viruses can cause AIDS however, pathogenic course with HIV-2 is considered to be longer. It is believed that the origin of the two viruses is from two African monkeys, the chimpanzee (*Pan troglodytes troglodytes*) for HIV-1<sup>7</sup> and the sooty mangabay (*Cercocebus atys*) for HIV-2.<sup>8</sup> The helper T lymphocytes which plays a central role in the regulation of the immune response are infected by HIV.<sup>9</sup> Due to the gradual depletion of these cells the patients become susceptible to bacterial, viral or fungal infections.<sup>10</sup> CD4 present at surface of the helper T-lymphocyte are the receptor for the HIV viral surface glycoprotein gp120.<sup>11,12</sup> The symptoms of the primary infection is characterized by rash, fever, headache, gastrointestinal disturbance, lymphadenopathy and pharyngitis.<sup>13,14</sup>

AIDS has become a pandemic which is escalating at an alarming rate.<sup>15</sup> UNAIDS and WHO estimate that 25 million people have died due to AIDS since its first report. It was estimated that about 39.5 million people were found to be living with HIV/AIDS and 4.3 million people were newly infected, and more than 2.9 million were dead due to this disease in 2006 alone.<sup>16</sup> The Indian scenario is also not very good. In 2008, it was estimated that 2.31 million people lived with this disease in India. India has an estimated 2.5 million infections (0.23% of population), making India the country with third largest population of HIV patients. Some recent studies have suggested that by the end of 2010, another 45 million people will be infected with HIV unless effective preventive measures are taken globally.<sup>17</sup>

## Structure of HIV Virus

HIV has unusual structure unlike other retroviruses. It is approximately spherical with a diameter of about 120 nm, almost 60 times smaller than a red blood cell still large enough to be a virus.<sup>18</sup> The lipid bilayer membrane enveloping HIV-1 virion consists of two type of glycoprotein attached to the surface, *viz.* the surface envelope protein (gp120) and the transmembrane envelope protein (gp41) (Figure 1).<sup>18</sup> The inner surface of the membrane is lined by matrix proteins while the capsid protein forms a cone-shaped core having two copies of identical, single-stranded RNA complexed with nucleocapsids and the replicative enzymes *viz.* reverse transcriptase, protease and integrase.<sup>19</sup>

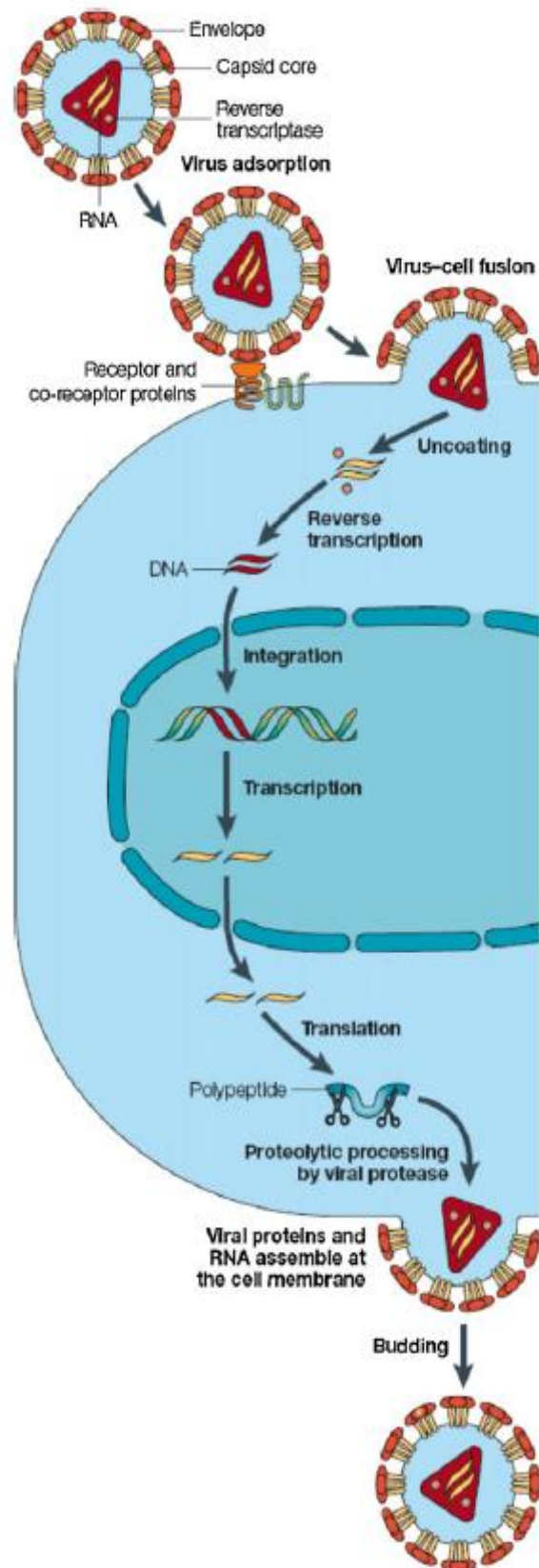


**Figure 1.** Organisation of the matured HIV-1 virion [Adapted from Wikipedia].

## Life Cycle of HIV

The life cycle of HIV virus is shown in the Figure 2. The genus of HIV is *Lentivirus*, part of the family of *Retroviridae* which carry genetic information in the form of RNA. The name retrovirus is given to this virus because in this virus DNA is transcribed from RNA which is completely contrary to the RNA is transcribed from

DNA. The life cycle of HIV-1 starts with binding of the envelope glycoprotein (gp120) of the virus with a surface CD4 receptor of the host cell. This is followed by the fusion of the viral and cellular membrane consequential in the “microinjection” of the capsid contents. A virally encoded reverse transcriptase present in the virus particle is responsible for conversion of the viral RNA to double-stranded DNA. Integration of this viral DNA into the cellular DNA is done by a virally encoded integrase, along with host cellular co-factors.<sup>20</sup> Once the infection of cell with virus takes place, either virus becomes active and replicates virus or becomes latent. The synthesis of viral proteins takes place due to the transcription of viral DNA into messenger RNA after the activation of host cell. Another virally encoded enzyme, HIV protease, is required to cleave a viral polyprotein precursor into individual mature proteins. The assembly of viral RNA and viral proteins at the cell surface generates new virions which then evolve from the cell and are released. The death of infected cells occurs<sup>21, 22</sup> due to the extensive cell damage caused by the destruction of the host’s genetic system and to the evolution and release of virions.



**Figure 2.** Life cycle of HIV virus<sup>20b</sup>.

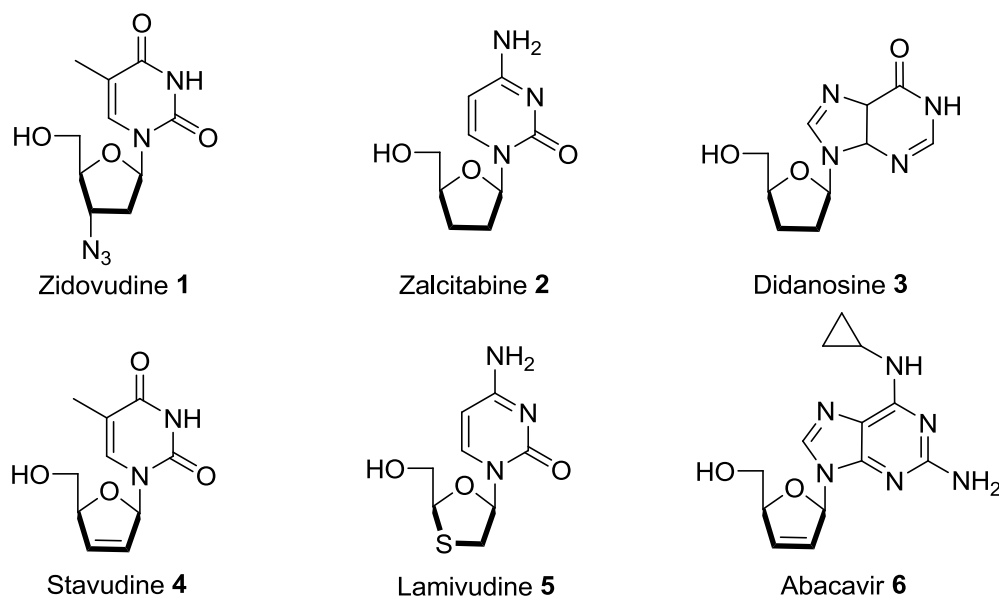
## Target for HIV

Every step in the HIV replication cycle could be considered as a potential target for anti-viral chemotherapy. The virus is an intracellular parasite, which depends on the metabolic pathways of the host cell. Therefore, most agents that block the replication of the virus are also harmful to the host cell. Hence, the numbers of practical targets for drug interventions are reduced. The process that is essential for the replication of the virus but not for the survival of the cell itself becomes the main target of anti-viral therapy.<sup>23</sup> With the knowledge about the replication cycle of the HIV-virus, scientists have focused their attentions on the various processes such as (a) viral binding to target cells, (b) virus cell fusion, (c) virus uncoating, (d) gene expression, (e) viral integration, (f) reverse transcription of genomic RNA, and (g) protease activity. So far, the last two strategies (f and g) have been established to be the most successful in the search for anti-HIV drugs.<sup>24</sup> These two targets will be described briefly in the following sections.

## Reverse Transcriptase Inhibitors

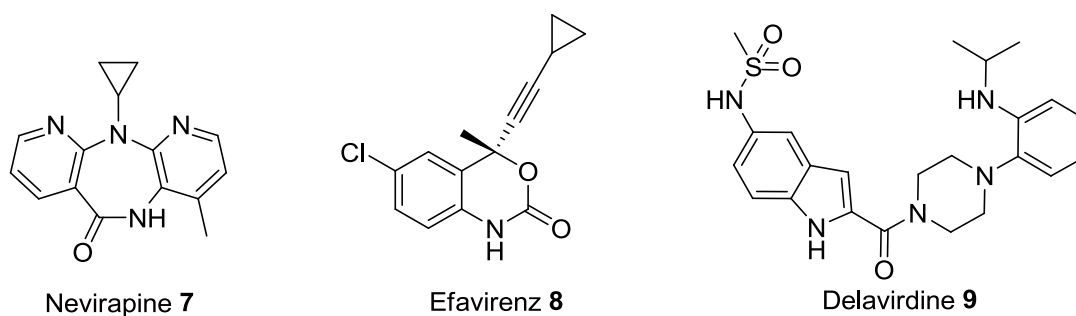
A reverse transcriptase (RT) is an enzyme recognised as RNA-dependent DNA polymerase that transcribes single-stranded RNA into double-stranded DNA. Thus, inhibition of RT prevents the formation of this double-stranded DNA that can be integrated in the host DNA. Reverse transcriptase inhibitors can be divided into two types, nucleoside (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI).<sup>25</sup> NRTI are analogues of the naturally occurring deoxynucleotides essential for the synthesis of viral DNA and they compete with the natural deoxynucleotides for incorporation into the growing viral DNA chain. They lack a 3'-hydroxyl group on the deoxyribose moiety, contrary to natural deoxynucleotides substrates. As a result, following incorporation of an NRTI the next incoming deoxynucleotide cannot form the next 5'-3' phosphodiester bond needed to extend the DNA chain. Thus, viral DNA synthesis is stopped and is known as chain termination. Due to the phosphorylation of nucleoside analogues by cellular kinases to triphosphates which mimic the natural substrate, the nucleotides.<sup>26</sup> The phosphorylated NRTI are incorporated into the growing DNA chain and terminate elongation.<sup>25</sup> Several NRTI have been approved

for clinical use (Figure 3). However, all are concomitant with side effects, such as bone marrow suppression, peripheral neuropathy and acute pancreatitis. Furthermore, prolonged treatment with these compounds imparts clinical resistance.



**Figure 3.** Nucleotide reverse transcriptase inhibitors

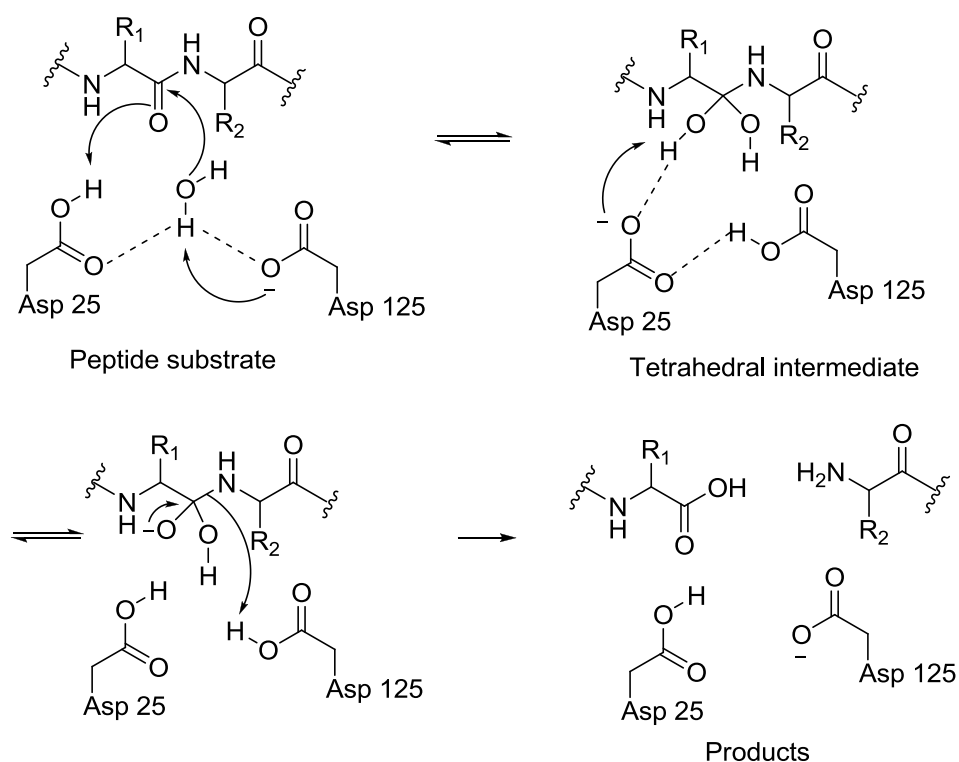
On the other hand, NNRTIs are compounds which interact with an allosteric site of HIV-1 RT and bind in a highly hydrophobic pocket of the enzyme and exhibit greater affinity for the enzyme-substrate complex than for the free enzyme.<sup>23</sup> NNRTIs are not incorporated into the viral DNA but instead inhibit the movement of protein domains of reverse transcriptase that are needed to carry out the process of DNA synthesis. The uniqueness of the hydrophobic allosteric site of HIV-1 RT brings a high selectivity index and a low toxicity of the NNRTs.<sup>25</sup> Following NNRTIs are being clinically used for the treatment of HIV infection (Figure 4).



**Figure 4.** Nonnucleotide reverse transcriptase inhibitors.

## HIV Protease Inhibitors

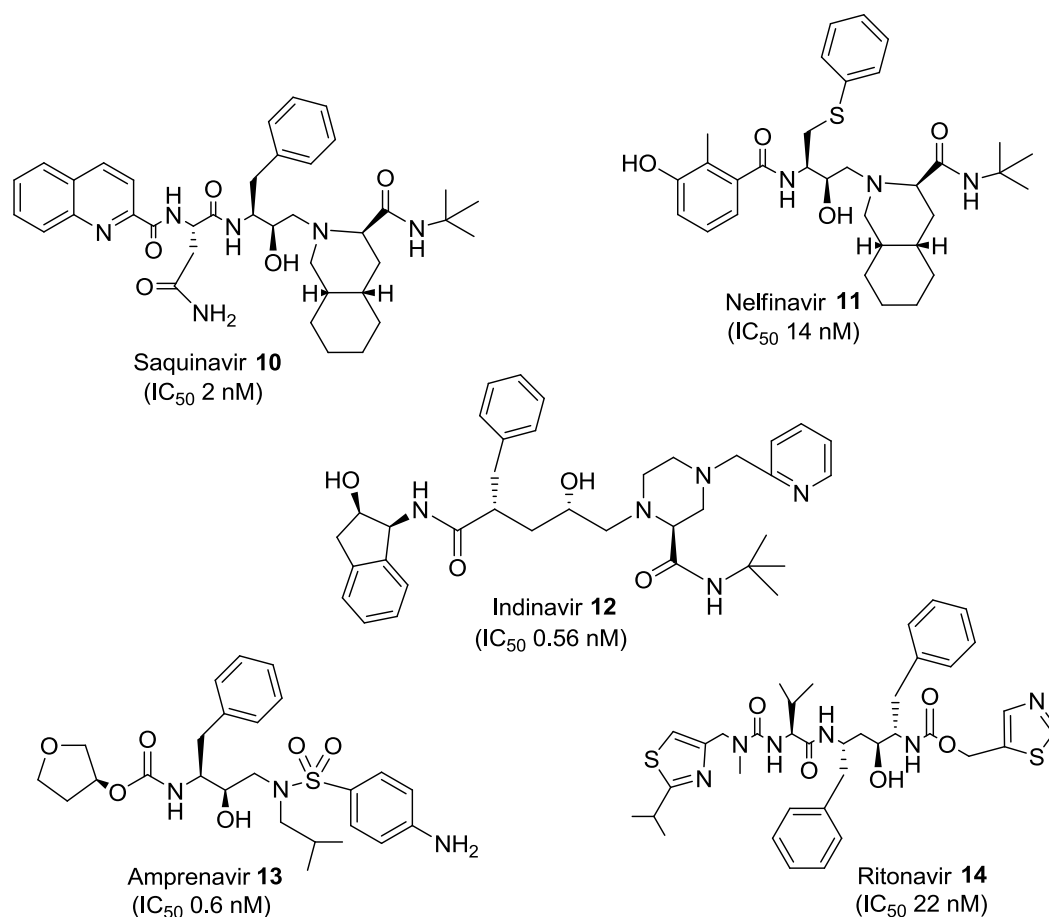
Cleavage of the Gag polyprotein precursor is crucial for the maturation of the HIV particles. Such cleavage is prohibited by a mutation in the protease region of the pol-gene. HIV-1 PR is one of the viral enzymes essential for the HIV-1 life cycle,<sup>27</sup> selectively cleaving viral polyproteins at specific peptide bonds. Figure 5 depicts how HIV-1 PR functions as a catalyst for hydrolyzing the viral gag-pol precursor proteins to produce structural proteins. Hydrolysis of the amide carbonyl group, by a water molecule accommodated between the side-chains of the aspartic acid residues 25 and 125, is believed to involve a tetrahedral intermediate.<sup>28</sup>



**Figure 5.** Catalytic mechanism of HIV protease.

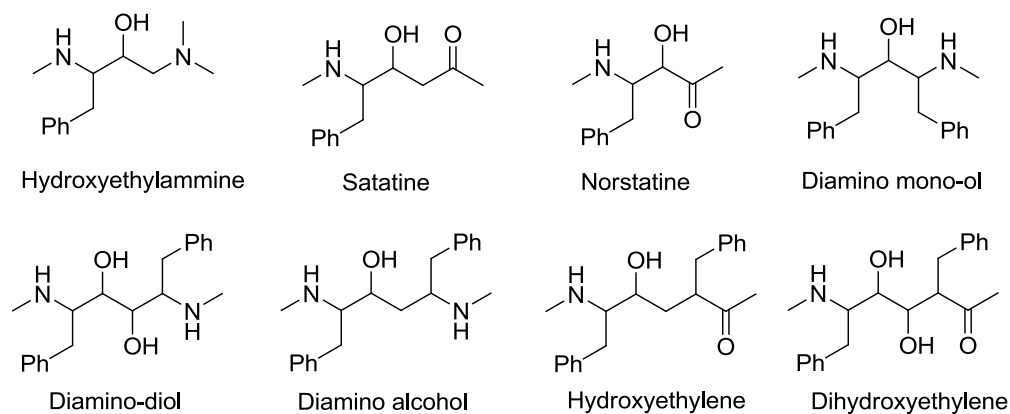
Hence, HIV protease was first suggested as a potential target for AIDS therapy by Kramer et al.<sup>29a</sup> Inhibition of HIV protease leads to the formation of immature non-infectious virions.<sup>29b</sup> Several potent HIV-1 protease inhibitors have been published.<sup>30,31</sup> Presently, there are many clinically approved protease inhibitors (Figure 6). Although, the present inhibitors are highly selective, they are also reported to possess some side effects such as lipodystrophy, hyperlipidaemia, insulin resistance<sup>32</sup> and emergence of resistant mutants upon prolonged use.<sup>33</sup>





**Figure 6.** Clinically approved HIV protease inhibitors.

Since HIV protease having a C<sub>2</sub>-symmetric homodimeric structure selectively cleaves the Phe-Pro (Tyr- Pro) moiety of the virus polyprotein, the rational design of inhibitors proved possible based on substrate models. Such substrate models consist of hydroxyethylene isosteres, diaminoalcohols and other related molecules shown in Figure 7.



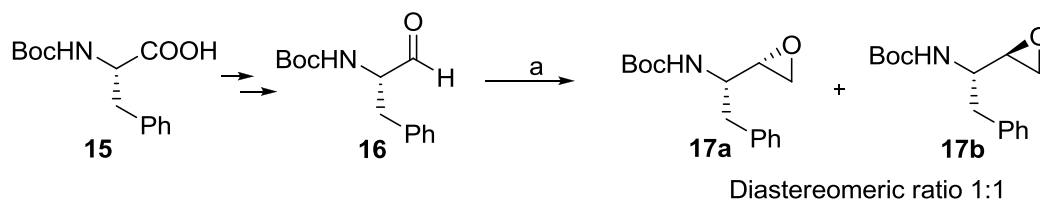
**Figure 7.** Substrate models for HIV protease inhibitors.

A non-hydrolysable hydroxyethylene or hydroxyethylamine (HEA) moiety has been used as the basic core for the development of clinical agents. Other non-cleavable transition-state isosteres have also been used including statine, norstatine, phosphinate, reduced amide, dihydroxyethylene and  $\alpha$ -keto. The HEA core is a good isostere replacement at the scissile bond that is believed to mimic the tetrahedral transition-state of the proteolytic reaction. Several inhibitors have been developed and most of the effective inhibitors contain HEA core structure to mimic the transition-state of the protease catalysis.

The HEA is one of the most important substrate models for the development of various HIV inhibitors and therefore, various methods for the synthesis of HEA isostere are known<sup>34</sup>, some of them are discussed below.

### 1. Evans Approach

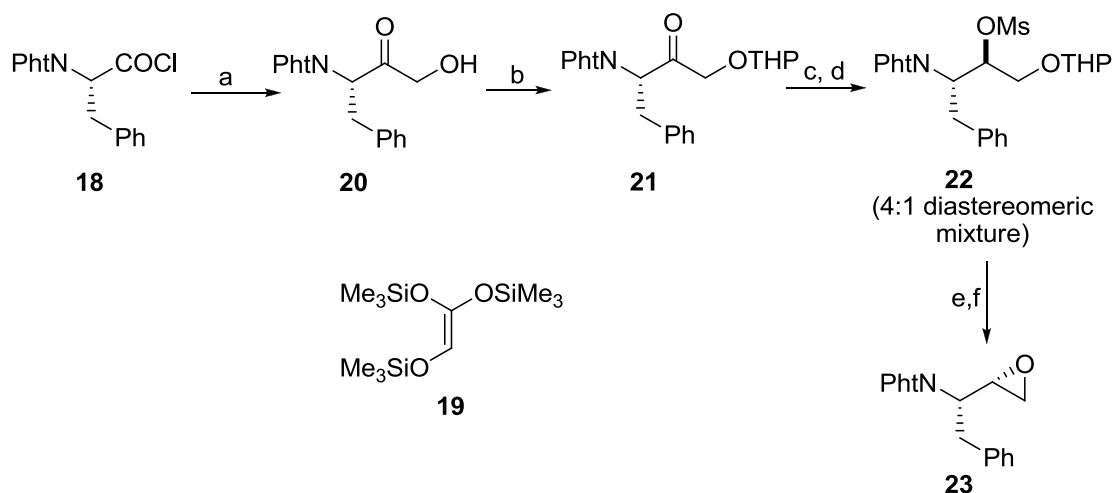
Evans *et al* reported the first synthesis of *erythro*-(2*S*,3*S*)-*N*-Boc aminoepoxide.<sup>34b,e,f</sup> Aldehyde **16** was prepared from *N*-Boc-phenylalanine **15** by reduction followed by oxidation. The aldehyde was subjected to the epoxidation reaction using dimethylsulfonium methylide gave a diastereomeric mixture of **17a** and **17b** in 46% yield in three steps (Scheme 1). The resulting epoxide was purified by crystallization from petroleum ether followed by recrystallization from hexane. Though this is not an industrially viable method from the viewpoint of diastereoselectivity, it is nevertheless noteworthy as a landmark synthesis of *N*-Boc aminoepoxide.



**Scheme 1.** Reagents and conditions: (a) dimethylsulfonium methylide, DMSO:THF 1:1, 46%.

## 2. Parkes's Synthesis

Parkes *et al.* reported a synthetic process for *erythro*-*N*-phthaloyl aminoepoxide **23** utilizing the hydroxymethyl ketone as a key intermediate (Scheme 2).<sup>34b,g,h</sup> Hydroxymethyl ketone **20** in turn was synthesized from the acid chloride of *N*-phthaloyl phenylalanine **18** using *tris*-(trimethylsilyloxy)ethane **19**. Protection with DHP gave **21** which was reduced with NaBH<sub>4</sub> followed by mesylation of hydroxyl group furnished compound **22** in a 4:1 diastereomeric mixture. The mesylate **22** was then converted to *erythro*-*N*-phthaloyl aminoepoxide **23** by treating it with *p*-toluenesulfonic acid followed by epoxidation with *t*-BuOK in DMF. This procedure requires rather lengthy reaction steps and the final product is obtained in low overall yield.

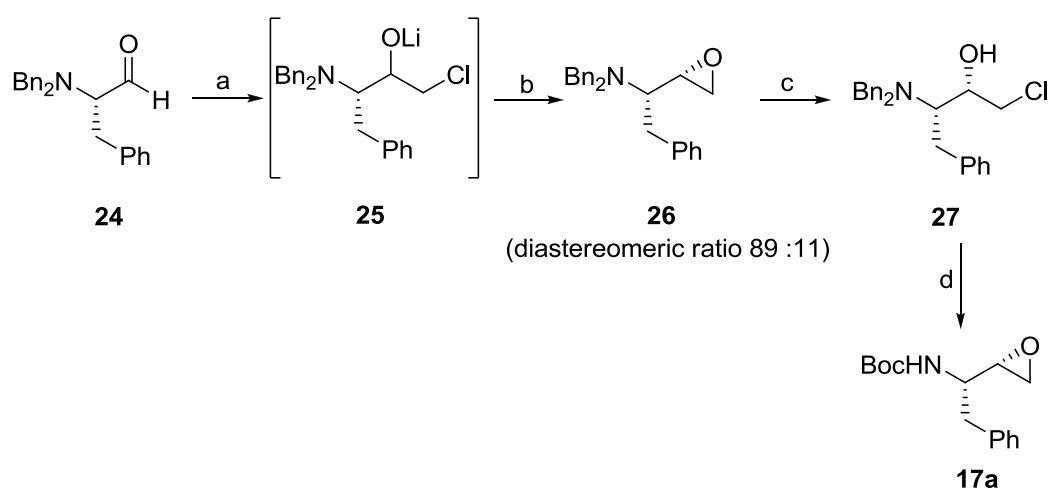


**Scheme 2.** Reagent and conditions: (a) **19**, 63%; (b) DHP; (c) NaBH<sub>4</sub>; (d) MsCl, Py; (e) PTSA, 29%; (f) *t*BuOK, 69%.

## 3. Beaulieu's Synthesis

Beaulieu *et al.* reported the stereoselective synthesis of the *N,N*-dibenzyl-protected aminoepoxide **26**.<sup>34b,i,1</sup> Chloromethylation of phenylalaninal **24** can be carried out in several ways.<sup>35</sup> In Scheme 3, one such chloromethylation is shown that includes treatment of ClCH<sub>2</sub>Li prepared *in situ* from BrCH<sub>2</sub>Cl with Li metal with aldehyde **24** to furnish *erythro*-*N,N*-dibenzyl aminoepoxide **26** as a major isomer in a ratio of 89:11 due to the influence of the steric hindrance of the dibenzylamino group. Since compound **26** could not be further purified, it was converted to the

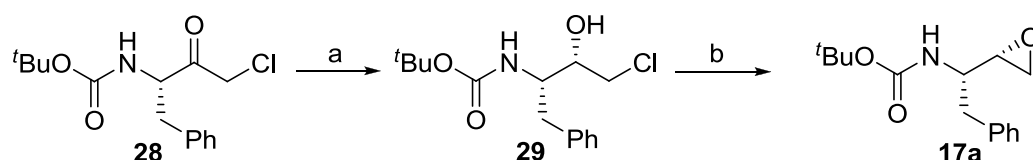
aminoalcohol **27** by means of treatment with 6 M HCl. The resulting *erythro*-aminoalcohol **27** could be purified by crystallization and then derivatized to the *erythro*-*N*-Boc aminoepoxide **17a** by hydrogenolysis of the dibenzyl groups followed by Boc protection and subsequent epoxidation under alkaline conditions. The synthesis reported by Beaulieu *et al.* might be considered as one of the best processes but suffers from some drawbacks such as requirement of an excess amount of Li metal and the relatively low yield.



**Scheme 3.** Reagents and conditions: (a) BrCH<sub>2</sub>Cl, Li shot, THF, -65°C; (b) rt; (c) 6 M HCl, 45% after three steps; (d) H<sub>2</sub>, Pd(OH)<sub>2</sub>/C, MeOH, 97%; (e) Boc<sub>2</sub>O/ TEA; (f) KOH/MeOH 78-96%.

#### 4. Synthesis via the *Erythro*-Selective Reduction of Halomethyl Ketone

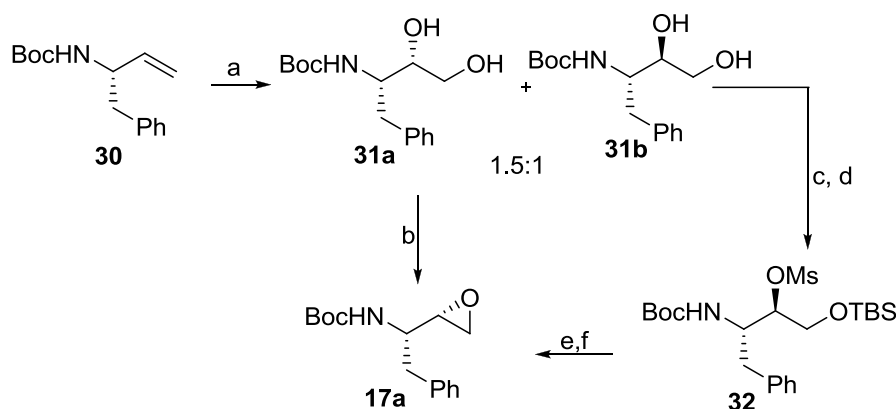
Synthesis of halomethyl ketone includes diazotization methods and various chloromethylenation methods.<sup>34b,35</sup> *N*-Alkoxy carbonyl  $\alpha$ -aminoalkyl  $\alpha$ -halomethyl ketones such as **28** may be selectively reduced with several agents such as NaBH<sub>4</sub> to give *erythro*-aminoalcohols **29**, which could be easily converted to epoxide **17a** by treatment with base.



**Scheme 4.** Reagents and conditions: (a) *Erythro*-selective reduction; (b) base.

## 5. Branalt's Synthesis

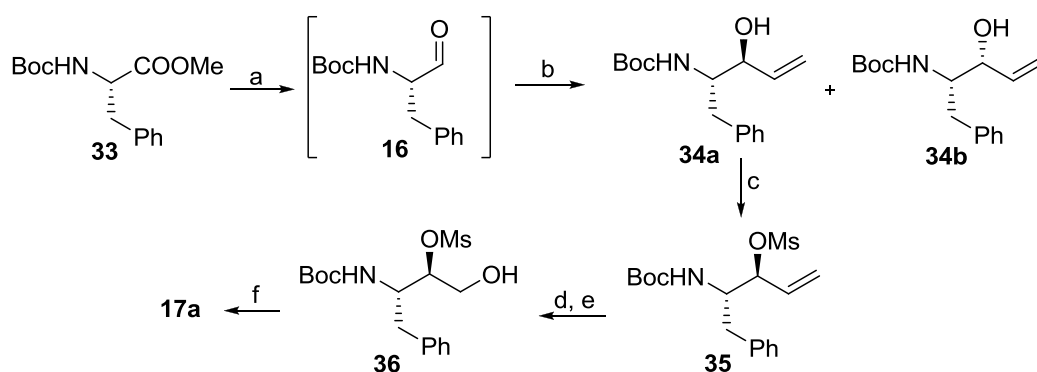
Branalt *et al.* reported synthesis of epoxide **17a** using dihydroxylation of the olefin **30** (Scheme 5).<sup>34b,o,p</sup> *N*-Boc-3-amino-4-phenylbutene **30** was treated with OsO<sub>4</sub>/NMO to afford amino-diols **31a** and **31b** in a diastereomeric ratio of 1.5:1. The diols **31a** and **31b** were separated by fractional crystallization from toluene. Both the isomers of diols could be transformed into the *erythro*-*N*-Boc aminoepoxide **17a**. The *erythro*-amino-diol **31a** was converted to **17a** in 95% yield using Mitsunobu reagent. *Threo*-amino-diol **31b** was also converted to the *erythro* *N*-Boc aminoepoxide **17a** by following a reaction sequence comprising of: (1) protection with TBSCl at the primary position; (2) mesylation of the secondary alcohol; (3) deprotection of the primary alcohol with a fluoride anion; and (4) epoxidation with NaH. The overall yield for these four steps was 72%.



**Scheme 5.** Reagents and conditions: (a) OsO<sub>4</sub>, NMO, 97%; (b) Ph<sub>3</sub>P, DEAD, 95%; (c) TBSCl, Imidazole; (d) MsCl, pyridine; (e) TBAF, THF; (f) NaH, THF, 72%.

## 6. Green's Synthesis

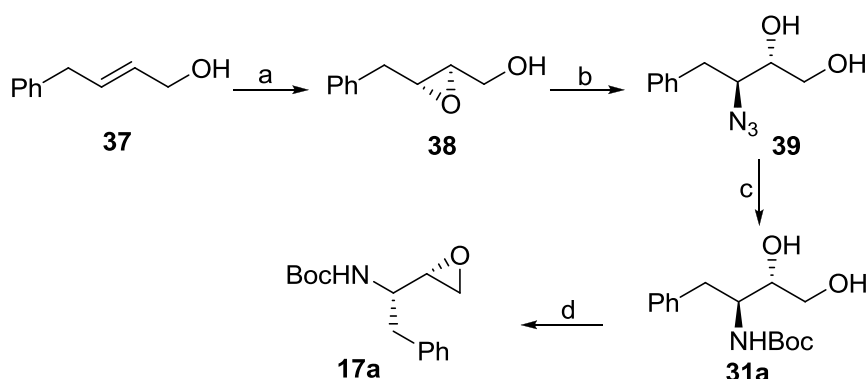
Green *et al.* reported another concise synthesis of isostere **17a** (Scheme 6).<sup>34b,q,r</sup> *N*-Boc-*L*-phenylalanine methyl ester **33** was treated with DIBAL-H at low temperature to furnish the aldehyde **16** followed by *in situ* addition of excess vinylmagnesium bromide to afford a 6:1 mixture of allylic alcohol **34a** and **34b** in 54% yield. Compound **34a** was then derivatized to the mesylate **35** followed by ozonolysis and *in situ* reduction with NaBH<sub>4</sub> to furnish alcohol **36**, which on reaction with base furnished *erythro*-*N*-Boc aminoepoxide **17a**.



**Scheme 6.** Reagents and conditions: (a) DIBAL, toluene,  $-78\text{ }^{\circ}\text{C}$ ; (b) vinyl magnesium bromide; (c) MsCl, DIPEA, DCM; (d)  $\text{O}_3$ , DCM, MeOH; (e)  $\text{NaBH}_4$ ; (f) NaH, THF, 85%.

## 7. Catusus's Synthesis

Synthesis of **17a** from the *erythro* epoxide **38** was suggested by Catusus *et al.* using Sharpless asymmetric epoxidation, ring opening by azide, followed by reduction and Boc protection (Scheme 7).<sup>34b,s</sup> The *erythro* amino-diol **31a** thus obtained was then converted to the *erythro*-*N*-Boc aminoepoxide **17a** under Mitsunobu conditions.



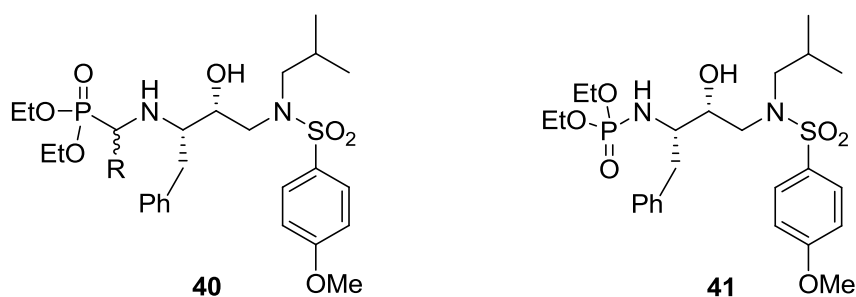
**Scheme 7.** Reagents and conditions: (a) Sharpless epoxidation, 88%; (b)  $\text{Ti}(\text{PrO})_2(\text{N}_3)_2$ ,  $\text{C}_6\text{H}_6$ ; (c)  $\text{H}_2$ , Pd/C,  $\text{Boc}_2\text{O}$ , EtOAc, 77% ; (d)  $\text{PPh}_3$ , DEAD, 75%.

## Present Work

HIV proteases are very important for the life cycle of HIV virus which possesses a  $\text{C}_2$ -symmetric homodimeric structure which selectively cleaves the Phe-Pro (Tyr-Pro) moiety of the virus protein.<sup>34b</sup> Inhibition of the HIV-protease is a well known strategy for the development of new anti-HIV agents.<sup>29,30</sup> HIV-protease cleaves the peptide bond *via* the tetrahedral intermediate in which water plays an important role.<sup>27,28</sup> Various inhibitors have been designed based on this transition-state known as

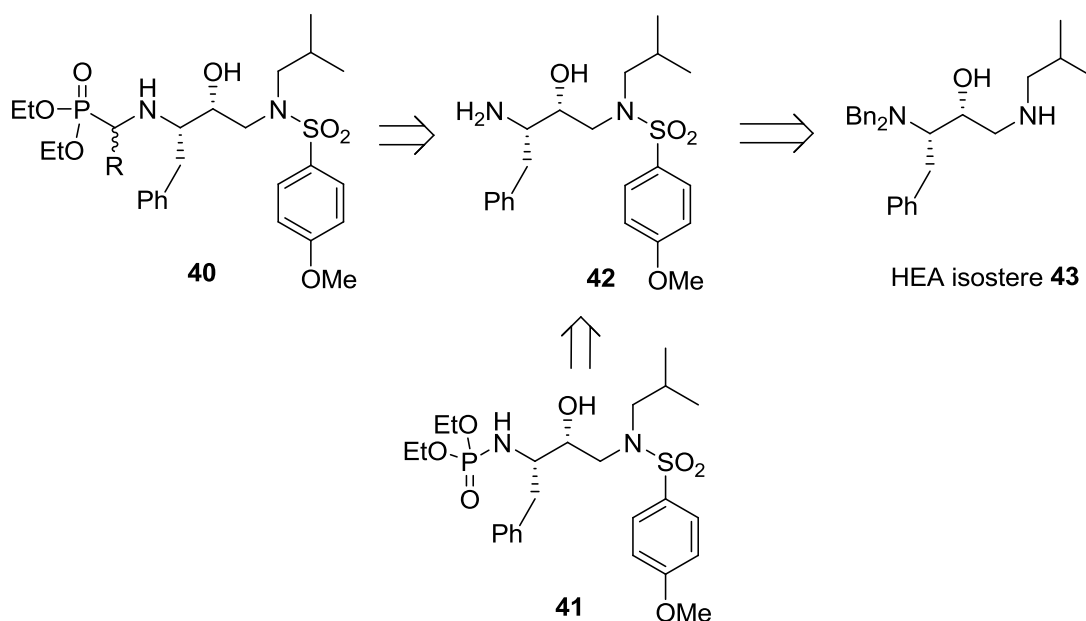
transition-state analogues.<sup>31</sup> Based on this concept of transition-state analogues, different substrate models were designed. Such substrate models consist of hydroxyethylene, diaminoalcohols, hydroxyethylamine (HEA), statine, norstatine, diamino-mono-ol, diamino diol, diamino alcohol, and dihydroxyethylene isosteres (Figure 7, page 54).<sup>34b</sup>

Out of these substrate models hydroxyethylamine (HEA) is an attractive motif due to its low molecular weight and it is the central core of several anti-HIV drugs *e.g.* amprenavir (Figure 5, page 53).<sup>31,34</sup> We opined that replacement of P<sub>2</sub> pocket group of the inhibitor with aminophosphonate could result in the more potent inhibitors as phosphonates are known to be stable under physiological conditions, do not react with enzymes like cholinesterase thus decreasing their toxicity, show good cell permeability and increases cellular accumulation and retention of drug compounds, thus improving their therapeutic and diagnostic value.<sup>36</sup> Keeping this in mind, we designed aminophosphonate derivatives of HEA isostere **40a-e** and phosphoramidate derivatives **41a** and **41b** (Figure 8) expecting that aminophosphonate will find its place in P<sub>2</sub> pocket of the enzyme and therefore, derivatives **40** and **41** could serve as potent anti-HIV agents.



**Figure 8.** Designed aminophosphonates and phosphoramidate derivatives of HEA isostere.

Aminophosphonate derivatives of HEA isostere **40a-e** and phosphoramidate derivatives **41a** and **41b** could be synthesized from corresponding amine **42** which in turn could be assessed from HEA isostere **43** as depicted in Scheme 8.



**Scheme 8.** Retrosynthesis of aminophosphonates **40** and phosphoramidate **41** derivatives of HEA isostere.

Several methods have been reported for the synthesis of HEA isostere **43** in the literature<sup>34</sup> however, these methods suffer from one or more drawbacks *viz.* low overall yields, lengthy steps and use of hazardous reagents such as lithium metal. Therefore, we carried out the present work which incorporates (i) a new and efficient method for the synthesis of hydroxyethylamine isostere and (ii) synthesis of  $\alpha$ -aminophosphonate and phosphoramidate derivatives of HEA isostere.

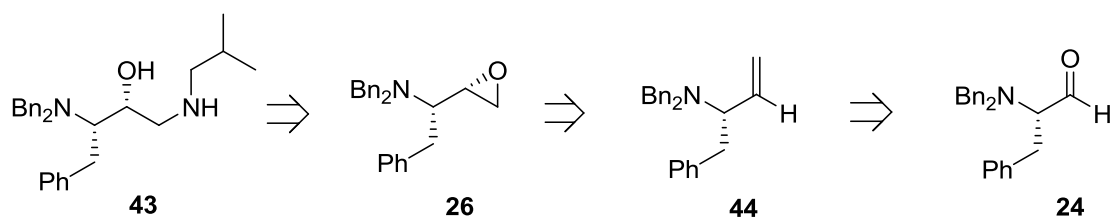
## Results and Discussion

### An Efficient Method for the Synthesis of HEA Isostere

#### Approach 1

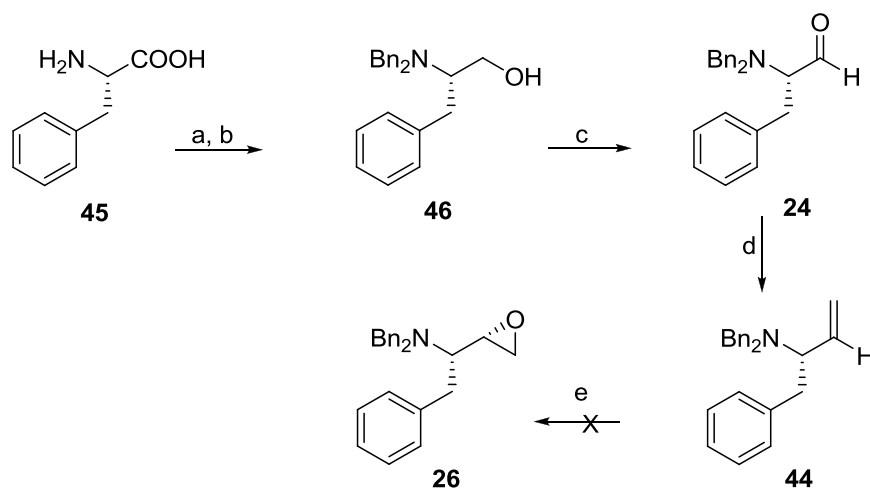
Retrosynthetically, HEA isostere **43** can be synthesized by the opening of epoxide **26** with isobutyl amine. The epoxide **26** can be prepared by the epoxidation of olefin **44** which in turn can be prepared from corresponding aldehyde **24** using Tebbe methylenation reaction (Scheme 9).



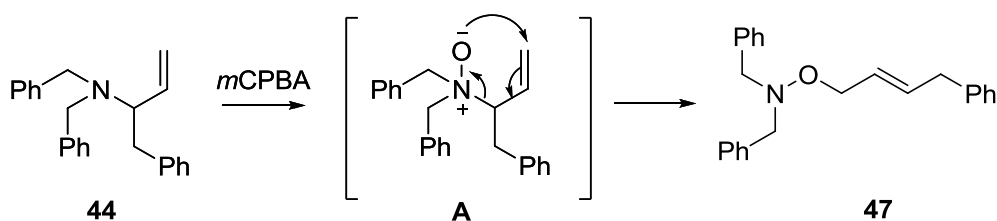


**Scheme 9.** Retrosynthesis of HEA isostere **43**.

We envisaged phenylalanine **45** as the starting material for the synthesis of compound **26** (Scheme 10). First, phenylalanine **45** was benzylated and subsequently reduced to dibenzyl-phenylalanol **46** which was oxidized using Swern oxidation conditions to form the aldehyde **24**.<sup>37</sup> The aldehyde **24** was subjected to Tebbe methylenation<sup>38</sup> to furnish olefin **44** in 63% yield. Reaction of olefin **44** with *m*CPBA did not give the corresponding epoxide **26** but it furnished a rearranged product which was identified as **47** (60%) by its spectral data. The formation of rearranged product **47** could be due to the fact that olefin **44** is a tertiary amine which underwent *N*-oxidation reaction followed by a rearrangement known as Meisenheimer rearrangement<sup>39</sup> (Scheme 11).



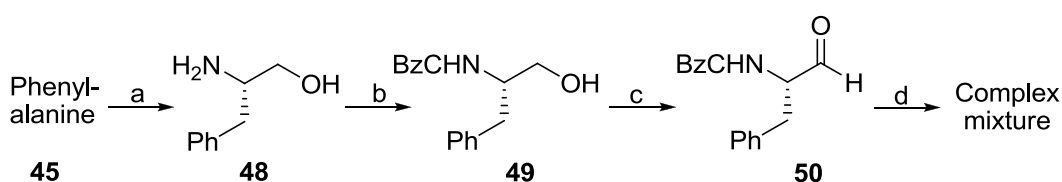
**Scheme 10.** Reagents and conditions: (a) NaOH, K<sub>2</sub>CO<sub>3</sub>, H<sub>2</sub>O, BnBr, reflux, 3 h; (b) LAH, Et<sub>2</sub>O, 0 °C, overnight, 60% after two step; (c) (COCl)<sub>2</sub>, DMSO, DCM, -78-0 °C, TEA, 1 h, 98%; (d) Tebbe reagent, THF, 0 °C, 30 min, 63%; (e) *m*CPBA, 0 °C, DCM.



**Scheme 11.** Meisenheimer rearrangement.

In order to access the desired epoxide **26**, it was decided to change the protecting group strategy for the amine group. Therefore, we thought that Cbz group could be effective as protecting group instead of dibenzyl group.

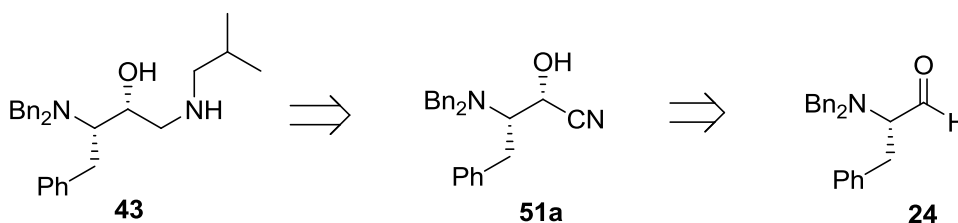
We began our synthesis by the LAH reduction of phenylalanine **45** to amino alcohol **48** followed by Cbz protection to furnish the compound **49**. The compound **49** was subjected to Swern oxidation to yield amino aldehyde **50**. Unfortunately, reaction of aldehyde **50** with Tebbe reagent<sup>38</sup> resulted in the formation of a complex mixture. This could be attributed to the instability of amino aldehydes and reactive nature of the Tebbe reagent (Scheme 12).



**Scheme 12.** Reagents and conditions: (a) LAH, THF, 0 °C to reflux, 73%; (b) CBZ-Cl, Bi(NO<sub>3</sub>)<sub>3</sub>·5H<sub>2</sub>O, MeOH, 30 min, 90%; (c) (COCl)<sub>2</sub>, DMSO, -78-0 °C, TEA, 1 h, 82%; (d) Tebbe reagent.

## Approach 2

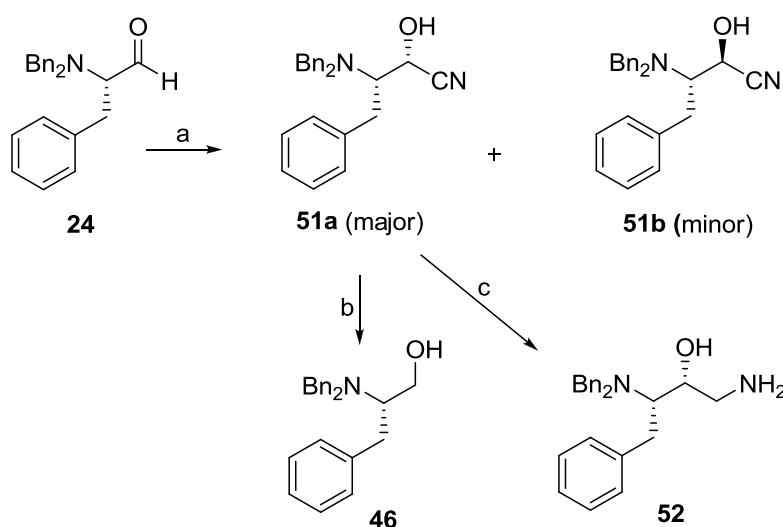
In the previous approach, we had attempted synthesis of isostere through epoxidation method which was not successful therefore, we thought of a new method for the synthesis of HEA isostere as depicted in Scheme 13. In this approach, HEA isostere **43** could be synthesized from cyanohydrin **51a** which in turn can be accessed from aldehyde **24**.



**Scheme 13.** Retrosynthesis of HEA isostere **43**.

Aldehyde **24** was subjected to Lewis acid catalyzed cyanohydrin formation using TMS-CN and BF<sub>3</sub>·Et<sub>2</sub>O to furnish **51a** as a major diastereomer.<sup>40</sup> The stereochemistries of compounds **51a** and **51b** were confirmed by their spectral data.

In compound **51a**, proton of  $\underline{C}HOH$  appearing at  $\delta$  3.98 ppm (d) showed a coupling constant of 5.4 Hz while that of **51b** ( $\delta$  4.25 ppm, d) exhibited higher coupling constant *i.e.* 8.1 Hz thereby indicating that the stereochemistries in compound **51a** and **51b** are (*S,S*) and (*R,R*), respectively which is in accordance with the reported literature data.<sup>41</sup> The cyanohydrin **51a** having the desired stereochemistry was subjected to LAH reduction (Scheme 14) at 0 °C however, decyanated alcohol **46** was obtained as a major product (60%). Finally, the synthesis of compound **52** was achieved by the reduction of cyanohydrin **51a** by *in situ* generated nickel boride albeit in low yield. In order to achieve the synthesis of desired isostere **43**, two more subsequent reactions on compound **52** *viz.* imine formation with isobutyraldehyde followed by reduction of imine with  $NaBH_4$  will be required to be performed. Since compound **52** was obtained in 50% yield and further two more reactions needed to be performed for the ultimate synthesis of isostere **43**, this scheme was found to be not interesting therefore we decided to develop a one-pot method.



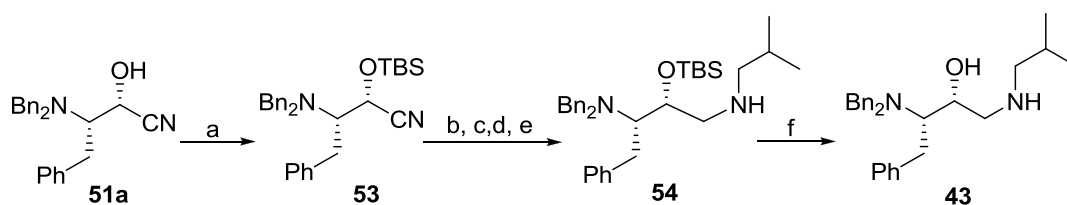
**Scheme 14.** Reagents and conditions: (a)  $TMSCN$ ,  $BF_3 \cdot Et_2O$ ,  $DCM$ ,  $-20$  °C, 2 h, 80%; (b)  $LAH$ ,  $THF$ ,  $0$  °C, 1 h, 60%; (c)  $NiCl_2 \cdot 6H_2O$ ,  $NaBH_4$ ,  $MeOH$ ,  $0$  °C, 1 h, 50%.

### Approach 3

#### One-pot reduction-transimination-reduction

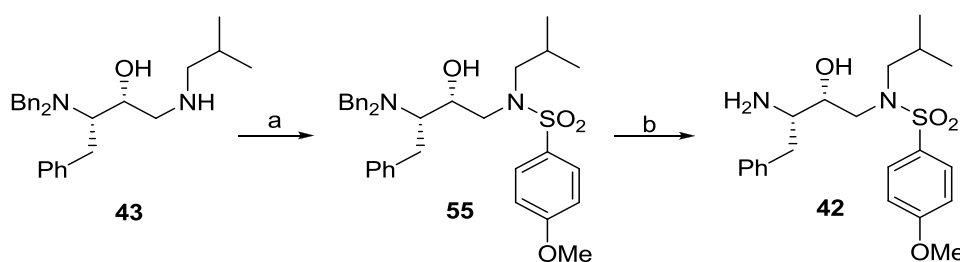
In the one-pot reduction-transimination-reduction approach, initially the cyano group is reduced by  $DIBAL-H$  at  $-78$  °C followed by reaction with methanolic

ammonium bromide which converts iminium aluminium complex into the free N-H imine. This primary imine is then converted into more stable secondary imine by transimination reaction.<sup>42</sup> Afterwards, reduction of secondary imine with NaBH<sub>4</sub> is carried out in the same pot to furnish the secondary amine. We decided to carry out synthesis of HEA isostere **43** following this approach. We began our synthesis with the TBS protection of cyanohydrin **51a** by treating it with TBSCl to furnish compound **53**. The reduction-transimination-reduction reaction was employed on protected cyanohydrin **53** (Scheme 15), amine **54** was obtained in good yield (75%). The TBS group was deprotected with TBAF in THF solution (1M) to furnish the desired isostere **43**. Thus, we were successful in developing a one-pot reduction-transimination-reduction approach for the synthesis of isostere **43**.



**Scheme 15.** Reagents and conditions: (a) TBS-Cl, Imidazole, DCM, rt, overnight, 93%. (b) DIBAL-H, Et<sub>2</sub>O, -78 °C, 3 h; (c) NH<sub>4</sub>Br in MeOH; (d) Isobutyl amine, 3 h; (e) NaBH<sub>4</sub>, 75% in four steps; (f) TBAF, THF, 85%.

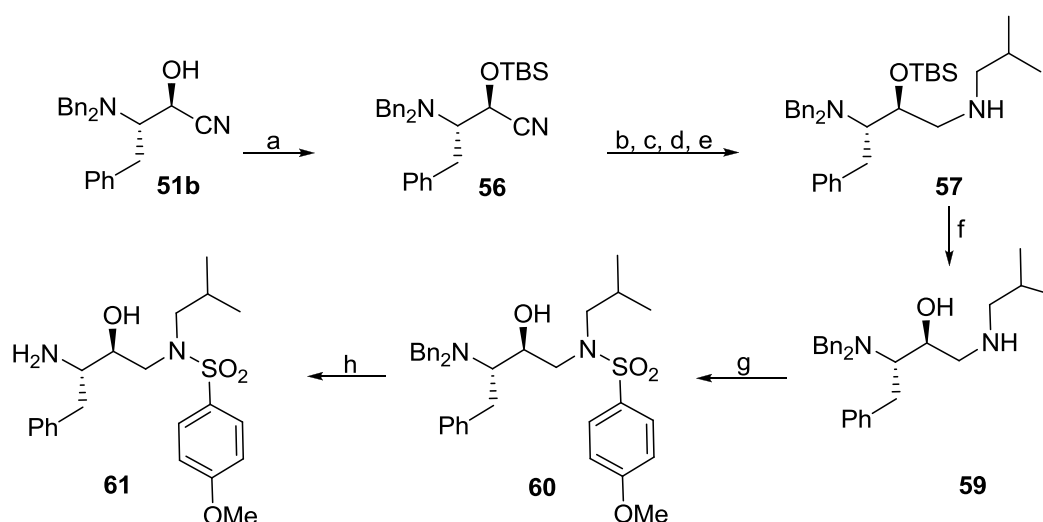
The HEA isostere **43** was subjected to *N*-sulfonylation reaction<sup>28r</sup> with aq. sodium carbonate in DCM to afford compound **55** which on benzyl deprotection with Pd(OH)<sub>2</sub>/C, H<sub>2</sub> furnished the free amine **42** (Scheme 16).



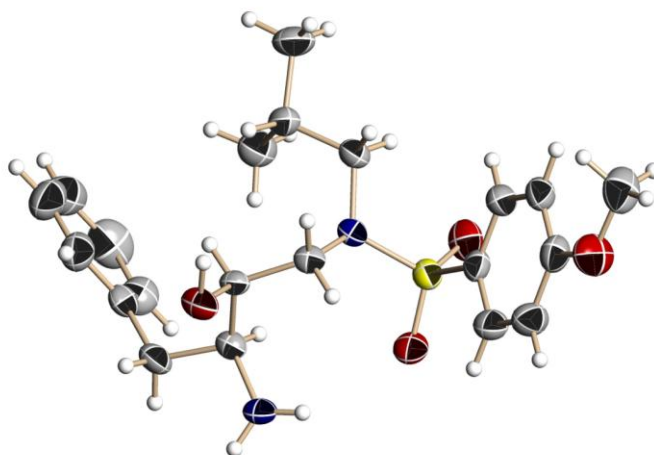
**Scheme 16.** Reagents and conditions: (a) 4-Methoxysulfonylchloride, DCM, aq. Na<sub>2</sub>CO<sub>3</sub>, 3 h, 71%; (b) Pd(OH)<sub>2</sub>/C, H<sub>2</sub>, 1 atm, overnight, 85%.

### Confirmation of the Stereochemistry of HEA Isostere **43** and **61**

The spectral data of the HEA isostere **43** was compared with that of known in the literature<sup>43</sup> and it was found to be in good agreement with the reported data. This clearly proved that the stereocentre of the hydroxyl group was  $\alpha$ -oriented. Moreover, when the similar set of reactions were performed on the minor diastereomer **51b** as depicted in Scheme 17, compound **61** was obtained as a crystalline solid. The X-ray crystal structure analysis of this compound **61** unambiguously proved the  $\beta$ -orientation of the hydroxyl group (Figure 9). This indirectly proves that the hydroxyl group of other isomer **42** to be  $\alpha$ -oriented.



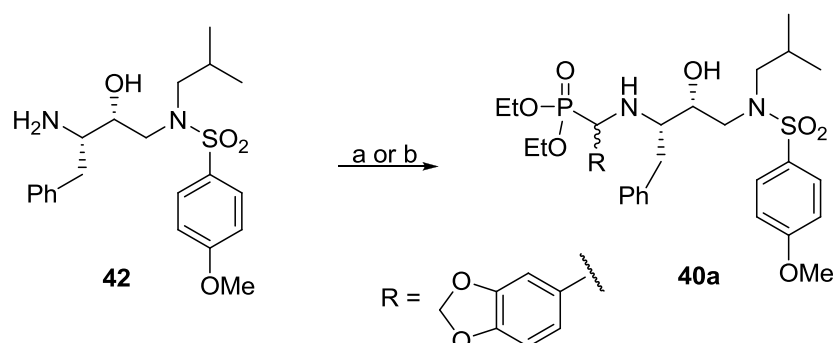
**Scheme 17.** Reagents and conditions: (a) TBDMS-Cl, Imidazole, DCM, rt, overnight, 85%; (b) DIBAL-H, Et<sub>2</sub>O, -78 °C, 3 h; (c) NH<sub>4</sub>Br in MeOH; (d) Isobutyl amine, 3 h; (e) NaBH<sub>4</sub>, overnight, 78% in four steps; (f) TBAF, THF, 81%; (g) 4-methoxysulfonylchloride, DCM, aq. Na<sub>2</sub>CO<sub>3</sub>, 3 h, 83%; (h) Pd(OH)<sub>2</sub>/C, H<sub>2</sub>, 1 atm, overnight, 80%.



**Figure 9.** ORTEP diagram of compound **61**.

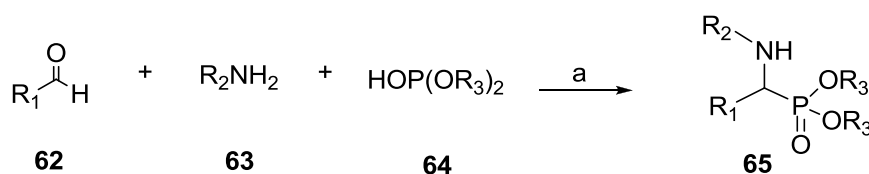
## Synthesis of $\alpha$ -Aminophosphonate Derivatives of HEA Isostere

Since we have already accomplished the synthesis of hydroxyethylamine isostere **43** and free amine **42**, our next aim was to synthesize its  $\alpha$ -aminophosphonate derivatives. For this purpose, we carried out Kabachnik-Fields reaction of the amine with piperonal following known literature methods<sup>44</sup> that include catalytic systems like  $\text{TaCl}_5$ ,  $\text{InCl}_3$ , and  $\text{Mg}(\text{ClO}_4)_2$  under various reaction conditions. However, none of the reported methods gave the corresponding product **40a** (Scheme 18). The reactions were limited up to the formation of imine and unreacted starting materials (~60%) were recovered even when the reaction was continued after 24 h. When reaction was repeated in the presence of Amberlite-IR 120 (acidic) under neat reaction condition, the traces of product formation was observed after 24 h. We carried out the same reaction under microwave irradiation and the corresponding product **40a** was obtained in good yield within 1 minute as a diastereomeric mixture.



**Scheme 18.** Reagents and conditions: (a)  $\text{TaCl}_5$  or  $\text{InCl}_3$  or  $\text{Mg}(\text{ClO}_4)_2$ , RCHO, diethyl phosphite; (b) RCHO, diethyl phosphite, MWI, Amberlite-IR 120 (acidic), 53%.

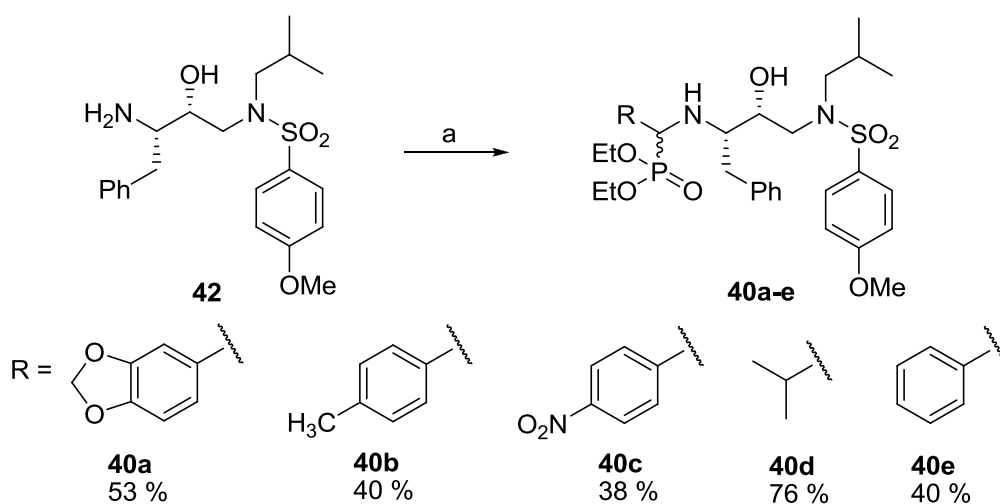
Most of catalytic systems reported for the Kabachnik-Fields reaction use aromatic aldehyde and aromatic-amine and there are very few reports for the reaction of aromatic aldehyde and aliphatic amine.<sup>45</sup> Hence we opined that Amberlite-IR 120 under microwave irradiation<sup>45d</sup> would be a good alternative for the synthesis of  $\alpha$ -aminophosphonate derivatives of isostere **43** using Kabachnik-Fields reaction (Scheme 19) (for detail discussions, please see Chapter 4, Section B).



**Scheme 19.** Reagents and conditions: (a) Amberlite IR 120 (acidic), MWI.

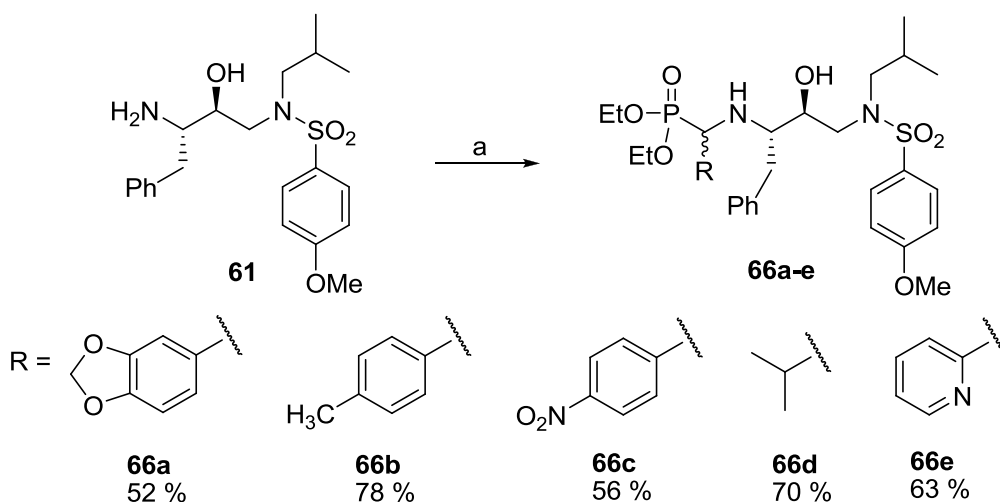
The free amine **42** was treated with diverse aldehydes and diethylphosphite under microwave irradiation in presence of Amberlite IR-120 as catalyst to furnish corresponding  $\alpha$ -aminophosphonates **40a-e** as mixtures of diastereomers (Scheme 20).

The diastereomeric ratio was calculated from the  $^1\text{H}$  NMR spectral data. These diastereomers were found to be inseparable by column chromatography as well as by preparative thin layer chromatography.



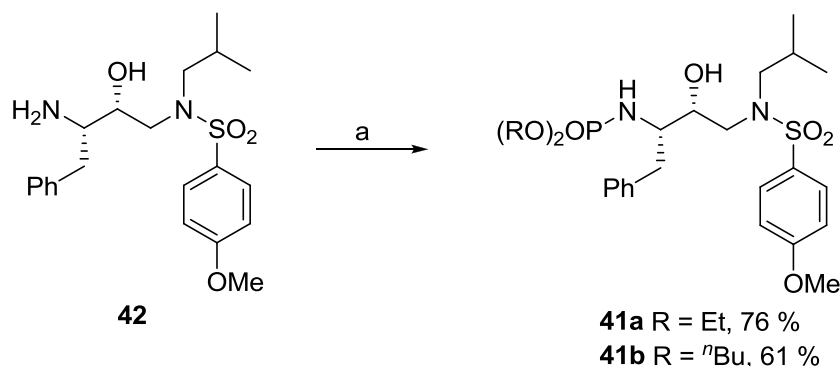
**Scheme 20.** Reagents and conditions: (a) RCHO, Diethyl phosphite, MWI, Amberlite-IR 120 (acidic).

Similarly,  $\alpha$ -aminophosphonate derivatives of HEA isostere **66a-e** having  $\beta$ -oriented hydroxyl group were also synthesized from amine **61** by employing the above mentioned method (Scheme 21).



**Scheme 21.** Reagents and conditions: (a) RCHO, Diethyl phosphite, MWI, Amberlite-IR 120 (acidic).

In addition to  $\alpha$ -aminophosphonate derivatives, it was decided to synthesize phosphoramidate derivatives **41a-b**. The amine **42** was subjected to the Atherton-Todd<sup>46</sup> reaction conditions (Scheme 22) with diethylphosphite or dibutylphosphite to furnish the desired phosphoramidate derivatives **41a-b**.



**Scheme 22.** Reagents and conditions: (a) DEP or DBP, CCl<sub>4</sub>, K<sub>2</sub>CO<sub>3</sub>, DCM.

### Biological activity

All the synthesized  $\alpha$ -aminophosphonate and phosphoramidate derivatives of HEA (**40a-e** and **66a-e**) and (**41a-b**), respectively were assayed for their biological activity against wild-type HIV-1 strain III<sub>B</sub> and HIV-2 strain ROD along with the double mutant strains RES056 (K103N + Y181C) according to the MTT method in MT-4 cells. The results of the bioassay of all the compounds expressed as IC<sub>50</sub>, CC<sub>50</sub> and SI (selectivity index) are summarized in Table 1. The drugs currently being used



in clinical treatment of HIV-1 infection, DDN/DDC and DMP266 were used as control.

**Table 1.** Bioassay of our synthesized compounds against HIV-1 in MT-4 cells.

Entry	Compound	Strain <sup>a</sup>	IC <sub>50</sub> <sup>b</sup> (µg/ml)	CC <sub>50</sub> <sup>c</sup> (µg/ml)	SI <sup>d</sup>
1	<b>40a</b>	III <sub>B</sub>	> 13.20	13.20	< 1
		ROD	> 13.20	13.20	< 1
2	<b>40b</b>	III <sub>B</sub>	> 2.47	2.47	< 1
		ROD	> 2.47	2.47	< 1
3	<b>40c</b>	III <sub>B</sub>	> 14.24	14.24	< 1
		ROD	> 14.24	14.24	< 1
4	<b>40d</b>	III <sub>B</sub>	> 11.52	11.52	< 1
		ROD	> 11.52	11.52	< 1
5	<b>40e</b>	III <sub>B</sub>	> 13.25	13.25	< 1
		ROD	> 13.25	13.25	< 1
6	<b>66a</b>	III <sub>B</sub>	> 13.73	13.73	< 1
		ROD	> 13.73	13.73	< 1
7	<b>66b</b>	III <sub>B</sub>	> 4.94	4.94	< 1
		ROD	> 4.94	4.94	< 1
8	<b>66c</b>	III <sub>B</sub>	> 3.94	3.94	< 1
		ROD	> 3.94	3.94	< 1
9	<b>66d</b>	III <sub>B</sub>	> 13.68	13.68	< 1
		ROD	> 13.68	13.68	< 1
10	<b>66e</b>	III <sub>B</sub>	> 13.93	13.93	< 1
		ROD	> 13.93	13.93	< 1
11	<b>41a</b>	III <sub>B</sub>	7.77	64.50	8
		RES056	7.40	64.50	9
		ROD	> 64.50	64.50	< 1
12	<b>41b</b>	III <sub>B</sub>	> 13.08	13.08	< 1
		RES056	> 13.08	13.08	< 1
13	DDN/DDC	III <sub>B</sub>	0.37	0.04	> 55
		ROD	0.49	0.16	> 41
14	DMP266	III <sub>B</sub>	0.0018	0.0001	> 1133
		RES056	0.17	0.01	> 12

<sup>a</sup>Strains: III<sub>B</sub>: Wild-type HIV-1; ROD: HIV-2; RES056: double mutant (K103N + Y181C).

<sup>b</sup>IC<sub>50</sub>: concentration of compound required to achieve 50% protection of MT-4 cells against HIV-induced cytotoxicity, as determined by the MTT method.

<sup>c</sup>CC<sub>50</sub>: concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method

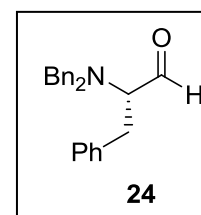
<sup>d</sup>SI: selectivity index (CC<sub>50</sub>/IC<sub>50</sub>).

The results summarized in Table 1 indicate that the phosphoramidate derivative of HEA **41a** (entry 11) was the most active amongst all the synthesized compounds against III<sub>B</sub> and RES056 strains. Interestingly, compound **41a** was inactive against HIV-2, it was found to be active against HIV-1 and double mutant strains RES056 with IC<sub>50</sub> values of 7.77 and 7.40 µg/ml and selectivity factors of 8 and 9, respectively. The synthesized  $\alpha$ -aminophosphonate derivatives of HEA (**40a-e** and **66a-e**) did not show any significant activity against HIV-1 (IIIB) as well as HIV-2 (ROD) with IC<sub>50</sub> values greater than the corresponding CC<sub>50</sub> values, rendering selectivity indexes less than 1.

## Experimental

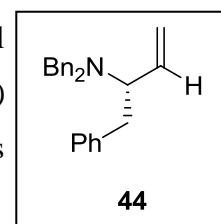
### (S)-2-(Dibenzylamino)-3-phenylpropanal (**24**).

Aldehyde **24** was synthesized from phenylalanine **45** following a known literature procedure.<sup>37</sup>



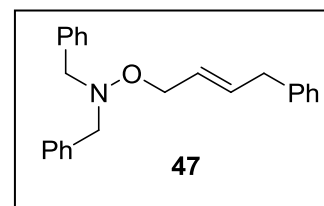
### (S)-N,N-Dibenzyl-1-phenylbut-3-en-2-amine (**44**).

A round-bottom flask equipped with a magnetic stirring bar and oil bubbler was charged with titanocene dichloride (250 mg, 1.0 mmol) and flushed with argon, and  $\text{AlMe}_3$  (2.0 M in PhMe, 1 mL) was added drop by drop. The resulting dark red mixture was stirred at rt with initial evolution of  $\text{CH}_4$  through the bubbler. Stirring was continued for 3 days. The flask was cooled to 0 °C to which was added a solution of **24** (329 mg, 1 mmol) in THF (4 mL). The reaction was allowed to warm up to rt and stirring was continued for 30 min. After completion of the reaction (TLC), the reaction mixture was diluted with diethyl ether (10 mL) followed by the slow addition of aq. NaOH (1 mL) till the gas evolution ceased. Then  $\text{Na}_2\text{SO}_4$  was added to the reaction mixture and it was filtered and the filtrate was concentrated to give the crude product which was purified by ethyl acetate: petroleum ether (2:98) to furnish olefin **44** (205 mg, 63%) as a colourless syrup;  $[\alpha]_{\text{D}}^{20} = +17.6$  (*c* 1,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ ): 3063, 3024, 2933, 2833, 2802, 1602, 1494, 1454, 1217, 1120  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.75-3.01 (ABX,  $J = 13.6$  Hz,  $J = 7.4$  Hz,  $J = 6.2$  Hz, 2H), 3.42-3.80 (AB,  $J = 13.9$  Hz, 4H), 3.32-3.43 (m, 1H), 5.00 (ddd,  $J = 2.0$  Hz,  $J = 1.0$  Hz,  $J = 17.2$  Hz, 1H), 5.22 (ddd,  $J = 10.4$  Hz,  $J = 0.6$  Hz,  $J = 2.0$  Hz, 1H), 5.85 (ddd,  $J = 17.2$  Hz,  $J = 10.4$  Hz,  $J = 2.3$  Hz, 1H), 7.00-7.23 (m, 15H),  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  38.4, 53.6, 62.2, 118.1, 125.9, 126.8, 128.1, 128.2, 128.6, 129.6, 135.9, 139.7, 140.2; MS (ESI):  $m/z$  328.2 ( $\text{M}+\text{H}$ )<sup>+</sup>. Anal. Calcd for  $\text{C}_{24}\text{H}_{25}\text{N}$ : C, 88.03; H, 7.70; N, 4.28. Found: C, 88.15; H, 7.77; N, 4.23.



**(E)-N,N-Dibenzyl-O-(4-phenylbut-2-enyl)hydroxylamine (47).**

To the solution of alkene **44** (80 mg, 0.244 mmol) in DCM (2 mL), *m*CPBA (46 mg, 0.27 mmol) was added at 0 °C. The reaction was allowed to stir at rt for 1 h. After completion of the reaction (TLC), water (5 mL) was added



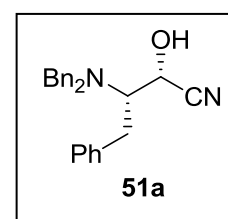
and the aqueous layer was extracted with DCM (3x5 mL). The combined organic extracts were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuum to afford crude product which was purified by flash chromatography using ethyl acetate: petroleum ether (3:97) to furnish the pure product **47** (50 mg, 60%) as a colourless syrup; IR (CHCl<sub>3</sub>): 3065, 3019, 2926, 2910, 1602, 1494, 1454, 1217, 1030 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 3.15 (d, *J* = 6.7 Hz, 2H), 3.65 (dd, *J* = 6.4, 6.6 Hz, 2H), 3.77 (s, 4H), 5.11-5.25 (m, 1H), 5.40-5.54 (m, 1H), 7.00-7.31 (m, 15H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ 38.8, 62.9, 74.3, 126.1, 127.1, 127.3, 128.2, 128.4, 128.6, 129.8, 133.5, 137.9, 140.1; MS (ESI): *m/z* 344.2 (M+H)<sup>+</sup>, 366.2 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>24</sub>H<sub>25</sub>NO: C, 83.93; H, 7.34; N, 4.08. Found: C, 83.83; H, 7.31; N, 4.15.

**Hydrocyanation of aldehyde (51a/51b).**

The mixture of aldehyde **24** (1.97 g, 6 mmol), trimethylsilylcyanide (750 μL, 7.2 mmol) and BF<sub>3</sub>.Et<sub>2</sub>O (1 mL, 7.2 mmol) in dry DCM (50 mL) was stirred at -20 °C for 2 h. After completion of the reaction (TLC), the reaction mixture was poured into H<sub>2</sub>O (50 mL) and the aqueous phase extracted with DCM (3x50 mL). The combined organic phases were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuum to yield crude product which was chromatographed on silica gel using ethyl acetate: petroleum ether (5:95) as eluant to furnish pure **51a** (2.1 g, 80%) and **51b** (0.21 g, 10%) as the major and minor products, respectively.

**(2S,3S)-3-(Dibenzylamino)-2-hydroxy-4-phenylbutanenitrile (51a).**

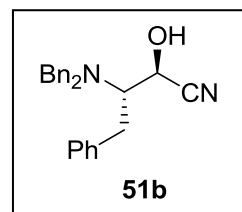
Yield: 80%; colourless syrup; [α]<sup>20</sup><sub>D</sub> = +48.4 (*c* 1, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>): 3421, 3064, 3020, 2928, 2841, 2401, 1602, 1521, 1494, 1454, 1375, 1215, 1074, 1028 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 2.94-3.01 (m, 1H), 3.18-3.34 (m, 2H), 3.52 (d, *J* = 13.1 Hz, 2H),



3.98 (d,  $J = 5.4$  Hz, 1H), 4.21 (d,  $J = 13.1$  Hz, 2H), 7.20-7.41 (m, 15H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  31.4, 54.6, 59.6, 61.1, 119.5, 127.1, 127.9, 128.9, 129.0, 129.1, 129.3, 136.8, 137.8; MS (ESI):  $m/z$  357.3 ( $\text{M}+\text{H}$ ) $^+$ , 379.3 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}$ : C, 80.87; H, 6.79; N, 7.86. Found: C, 80.95; H, 6.83; N, 7.73.

**(2R,3S)-3-(Dibenzylamino)-2-hydroxy-4-phenylbutanenitrile (51b).**

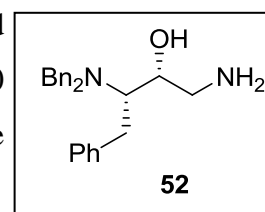
Yield: 10%; colourless syrup;  $[\alpha]_{\text{D}}^{20} = +47.0$  ( $c$  1,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ ): 3400, 3020, 2400, 1602, 1521, 1495, 1454, 1375, 1215, 1074, 1028  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.95-3.09 (m, 2H), 3.25-3.33 (m, 1H), 3.42-3.89 (AB,  $J = 13.3$  Hz, 4H), 4.25



(d,  $J = 8.1$  Hz, 1H), 7.15-7.40 (m, 15H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  32.4, 54.2, 61.8, 62.5, 119.2, 127.1, 127.7, 128.8, 128.9, 129.0, 129.4, 138.0, 138.1; MS (ESI):  $m/z$  357.3 ( $\text{M}+\text{H}$ ) $^+$ , 379.3 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}$ : C, 80.87; H, 6.79; N, 7.86. Found: C, 80.93; H, 6.59; N, 7.75.

**(2S,3S)-1-Amino-3-(dibenzylamino)-4-phenylbutan-2-ol (52)**

To a suspension of cyanohydrin **51a** (150 mg, 0.42 mmol) and  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  (200 mg, 0.84 mmol) in MeOH (3 mL),  $\text{NaBH}_4$  (160 mg, 4.2 mmol) was added slowly at 0 °C. The reaction mixture was stirred at rt for 1 h. After the completion of reaction (TLC),

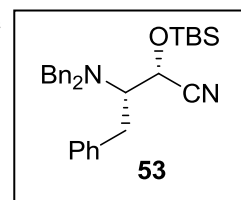


aq. HCl (3 N) was added till the black ppt. dissolved. The reaction mixture was evaporated to dryness under reduced pressure. Residue was dissolved in water and was made alkaline using aq. NaOH (1 N) and the product was extracted with ethyl acetate (3x10 mL), dried over  $\text{Na}_2\text{SO}_4$  and concentrated in vacuum to give crude product which was chromatographed over silica gel using ethyl acetate as eluant to furnish product **52** (75 mg, 50%); colourless syrup;  $[\alpha]_{\text{D}}^{20} = +6.55$  ( $c$  1.1,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ ): 3350, 3019, 1602, 1495, 1453, 1216  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.43-2.55 (m, 1H), 2.67-2.84 (m, 2H), 2.95-3.15 (m, 1H), 3.35-3.88 (m, 4H), 3.46 (bs, 2H), 3.96-4.18 (m, 2H), 7.15-7.34 (m, 15H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  32.7, 44.9, 54.6, 61.7, 71.8, 125.8, 127.0, 128.3, 128.9, 129.6, 139.9, 141.6; MS (ESI):  $m/z$

361.2 (M+H)<sup>+</sup>; Anal. Calcd for C<sub>24</sub>H<sub>28</sub>N<sub>2</sub>O: C, 79.96; H, 7.83; N, 7.77. Found: C, 80.06; H, 7.95; N, 7.68.

**(2*S*,3*S*)-2-(*Tert*-butyldimethylsilyloxy)-3-(dibenzylamino)-4-phenylbutanenitrile (53).**

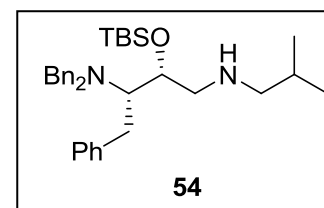
To the solution of cyanohydrin **51a** (20 g, 57 mmol) and imidazole (10 g, 143 mmol) in DCM (100 mL), TBSCl (13 g, 85 mmol) was added at 0 °C and the reaction mixture was stirred overnight at rt. After completion of the reaction (TLC), water (100 mL) was



added and the product was extracted with DCM (3x100 mL). The combined DCM layers were dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness under reduced pressure to give crude product which was chromatographed over silica gel using ethyl acetate: petroleum ether (5:95) as eluant to furnish product **53** (25 g, 93%); colourless syrup; [α]<sub>D</sub><sup>20</sup> = -34.23 (c 0.95, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>): 3063, 2955, 2858, 2357, 1602, 1494, 1469, 1369, 1263, 1122 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ (ppm) 0.00 (s, 3H), 0.06 (s, 3H), 0.78 (s, 9H), 2.87-2.91 (m, 2H), 3.22-3.32 (m, 1H), 3.51-3.67 (m, 4H) 4.36 (d, *J* = 5.8 Hz, 1H), 7.03-7.16 (m, 15H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ -5.2, -5.0, 18.1, 25.7, 33.2, 55.0, 63.3, 63.6, 120.0, 126.5, 127.2, 128.3, 128.5, 128.8, 129.6, 139.0, 139.2; MS (ESI): *m/z* 471.9 (M+H)<sup>+</sup>, 493.9 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>30</sub>H<sub>38</sub>N<sub>2</sub>OSi: C, 76.55; H, 8.14; N, 5.95. Found: C, 76.65; H, 8.20, N, 5.86.

**(2*S*,3*S*)-2-(*Tert*-butyldimethylsilyloxy)-3-(dibenzylamino)-4-phenylbutanenitrile (54).**

To a cooled solution (-78 °C) of cyanohydrin **51a** (470 mg, 1 mmol) in dry ether (8 mL), 1 M DIBAL-H solution in hexane (2.5 mL, 2.5 mmol) was added. After stirring at -78 °C for 3 h, NH<sub>4</sub>Br (240 mg) in dry methanol (4 mL) was

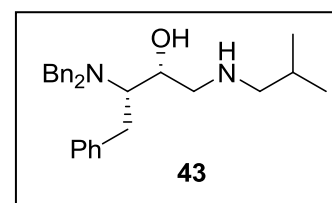


added. The cooling bath was removed and isobutyl amine (500 μL, 5 mmol) was added. Stirring was continued for another 3 h at rt. The mixture was cooled in an ice bath and NaBH<sub>4</sub> (74 mg, 2 mmol) was added in three portions. The reaction mixture was stirred overnight at room temperature. Water (20 mL) was added and product was extracted with ether (3x25 mL). The combined ether layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to furnish a crude product which was chromatographed

using ethyl acetate: petroleum ether (5:95) to afford product **54** (398 mg, 75%); colourless syrup;  $[\alpha]_{\text{D}}^{20} = -10.95$  ( $c$  1.0,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ ): 3063, 3018, 2955, 2856, 1602, 1494, 1454, 1361, 1255, 1215, 1120  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.00 (s, 3H), 0.01 (s, 3H), 0.67 (d,  $J = 6.6$  Hz, 3H), 0.69 (d,  $J = 6.7$  Hz, 3H), 0.80 (s, 9H), 1.32-1.52 (m, 1H), 2.03-2.21 (m, 2H), 2.43-2.48 (m, 2H), 2.68-2.89 (m, 2H), 3.01-3.09 (m, 1H), 3.47-3.65 (AB,  $J = 8.2$  Hz, 4H), 3.95-4.02 (m, 1H), 7.01-7.12 (m, 15H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  -4.2, -3.2, 18.3, 20.7, 20.8, 26.1, 28.4, 32.6, 54.6, 54.7, 58.3, 62.0, 72.2, 125.6, 126.7, 128.1, 128.8, 129.8, 140.3, 142.0; (ESI):  $m/z$  531.8 ( $\text{M}+\text{H}$ ) $^+$ , 553.8 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{34}\text{H}_{50}\text{N}_2\text{OSi}$ : C, 76.93; H, 9.49; N, 5.28. Found: C, 76.79; H, 9.60; N, 5.33.

**(2R,3S)-3-(Dibenzylamino)-1-(isobutylamino)-4-phenylbutan-2-ol (43).**

To the solution of **54** (150 mg, 0.28 mmol) in THF (1 mL), TBAF in THF (1 mL, 1.0 mmol) was added. The reaction mixture was stirred at rt. After completion of the reaction (TLC), THF was evaporated under reduced pressure.

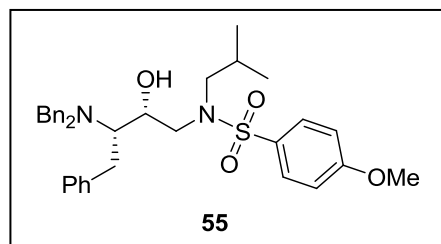


The residue was purified by silica gel column chromatography using EtOAc-pet-ether (2:3) as eluant to obtain **43** (100 mg, 85%); colourless syrup;  $[\alpha]_{\text{D}}^{20} = +4.71$  ( $c$  1.05,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ ): 3371, 3019, 2960, 2872, 2806, 1602, 1494, 1454, 1215  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.86 (d,  $J = 6.7$  Hz, 3H), 0.88 (d,  $J = 6.6$  Hz, 3H), 1.62-1.69 (m, 1H), 2.28-2.36 (m, 1H), 2.37-2.42 (m, 1H), 2.46-2.52 (m, 1H), 2.74-2.90 (m, 2H), 2.98-3.04 (m, 2H), 3.24 (bs, 2H), 3.57-3.73 (AB,  $J = 13.9$  Hz, 4H), 3.86-3.91 (m, 1H), 7.08-7.32 (m, 15H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  20.5, 28.1, 32.7, 53.0, 54.6, 57.2, 62.3, 68.6, 125.8, 126.9, 128.2, 128.3, 128.9, 129.7, 139.9, 141.5; (ESI):  $m/z$  417.5 ( $\text{M}+\text{H}$ ) $^+$ , 439.5 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{28}\text{H}_{36}\text{N}_2\text{O}$ : C, 80.73; H, 8.71; N, 6.72. Found: C, 80.79; H, 8.75; N, 6.77.

***N*-((2R,3S)-3-(Dibenzylamino)-2-hydroxy-4-phenylbutyl)-*N*-isobutyl-4-methoxybenzenesulfonamide (55).**

To the solution of **43** (200 mg, 0.48 mmol) in DCM (2 mL), a solution of  $\text{Na}_2\text{CO}_3$  (81 mg, 0.77 mmol) in water (500  $\mu\text{L}$ ) was added to it at 0  $^\circ\text{C}$ . To this stirred solution, a solution of *p*-methoxysulfonyl chloride (99 mg, 0.48 mmol) in DCM (1 mL) was added

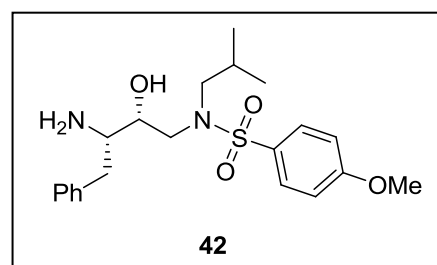
at 0 °C. The reaction was allowed to warm upto rt. After completion of the reaction (TLC), water (5 mL) was added and the product was extracted with DCM (3x10 mL), the combined organic layers were washed with brine (10 mL), dried over



$\text{Na}_2\text{SO}_4$  and concentrated in vacuum to obtain the crude product which was chromatographed over silica gel using ethyl acetate: petroleum ether (1:9) as eluant to give **55** (200 mg, 71%); colourless syrup;  $[\alpha]_{\text{D}}^{20} = -1.44$  (*c* 1.0, MeOH); IR ( $\text{CHCl}_3$ ): 3479, 3019, 2966, 1597, 1496, 1215, 1153, 1028  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.84 (d, *J* = 6.6 Hz, 3H), 0.94 (d, *J* = 6.6 Hz, 3H), 1.63 (bs, 1H), 1.77-1.82 (m, 1H), 2.65-2.95 (m, 4H), 2.99-3.08 (m, 3H), 3.60-3.76 (AB, *J* = 13.9 Hz, 4H), 3.87 (s, 3H), 4.07-4.17 (m, 1H), 6.92 (d, *J* = 8.9 Hz, 2H), 7.12-7.31 (m, 15H), 7.58 (d, *J* = 8.9 Hz, 2H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  19.9, 20.2, 27.3, 32.1, 54.6, 55.5, 55.6, 58.8, 62.4, 70.6, 114.2, 125.9, 126.9, 128.2, 128.3, 128.7, 129.5, 129.8, 130.6, 139.9, 141.0, 162.9; (ESI): *m/z* 587.5 ( $\text{M}+\text{H}$ )<sup>+</sup>, 589.5 ( $\text{M}+\text{Na}$ )<sup>+</sup>, 609.5 ( $\text{M}+\text{K}$ )<sup>+</sup>; Anal. Calcd for  $\text{C}_{35}\text{H}_{42}\text{N}_2\text{O}_4\text{S}$ : C, 71.64; H, 7.21; N, 4.77. Found: C, 71.74; H, 7.35; N, 4.83.

#### ***N*-((2*R*,3*S*)-3-Amino-2-hydroxy-4-phenylbutyl)-*N*-isobutyl-4-methoxybenzenesulfonamide (**42**)**

A mixture of **55** (500 mg, 0.85 mmol) and catalytic amount of  $\text{Pd}(\text{OH})_2$  in methanol (5 mL) were subjected to hydrogenation at 1 atm for 24 h at rt. The reaction mixture was filtered through celite and the filtrate was concentrated in vacuo.



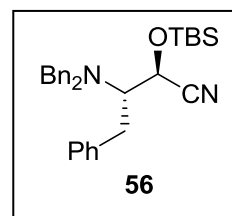
The residue was purified by silica gel column chromatography using a mixture of petroleum ether: ethyl acetate (3:7) to furnish **42** (293 mg, 85%); colourless syrup;  $[\alpha]_{\text{D}}^{20} = +16.28$  (*c* 0.90,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ ): 3479, 3018, 2968, 1597, 1496, 1334, 1261, 1215, 1153  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.88 (d, *J* = 6.7 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 3H), 1.82-1.96 (m, 1H), 2.48-2.54 (m, 1H), 2.81-3.03 (m, 3H), 3.07-3.35 (m, 3H), 3.73-3.76 (m, 1H), 3.86 (s, 3H), 6.98 (d, *J* = 8.9 Hz, 2H), 7.19-7.32 (m, 5H), 7.75 (d, *J* = 8.9 Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  19.9, 20.2, 39.1, 52.6, 55.6, 55.7, 58.5, 73.1, 114.3, 126.4, 128.6, 129.3, 129.5, 130.3, 138.9, 162.9; (ESI):



$m/z$  407.4 (M+H)<sup>+</sup>, 429.4 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>21</sub>H<sub>30</sub>N<sub>2</sub>O<sub>4</sub>S: C, 62.04; H, 7.44; N, 6.89. Found: C, 62.09; H, 7.53; N, 6.79.

**(2*R*,3*S*)-2-(Tert-butyldimethylsilyloxy)-3-(dibenzylamino)-4-phenylbutanenitrile (56).**

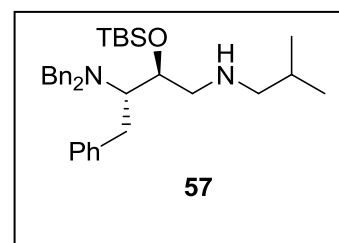
Title compound **56** was prepared starting from the cyanohydrin **51b** as per the procedure outlined for the preparation of protected cyanohydrin **53**. Yield: 85%; colourless syrup;  $[\alpha]_D^{20} = -13.9$  (*c* 1.0, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>): 3021, 2959, 2931, 2859, 2401, 1602, 1471, 1495, 1454, 1364, 1257, 1216, 1108 cm<sup>-1</sup>; <sup>1</sup>H NMR (200



MHz, CDCl<sub>3</sub>):  $\delta$  0.00 (s, 3H), 0.13 (s, 3H), 0.85 (s, 9H), 2.95-3.10 (m, 3H), 3.53-3.68 (m, 3H), 4.05-4.12 (m, 1H), 4.37-4.38 (m, 1H), 7.08-7.35 (m, 15H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  -5.4, -5.1, 18.1, 25.6, 30.8, 55.3, 62.4, 64.7, 119.8, 127.1, 128.3, 128.6, 128.8, 128.9, 129.3, 138.9, 139.3; MS (ESI):  $m/z$  471.9 (M+H)<sup>+</sup>, 493.9 (M+Na)<sup>+</sup>, 509.8 (M+K)<sup>+</sup>; Anal. Calcd for C<sub>30</sub>H<sub>38</sub>N<sub>2</sub>OSi: C, 76.55; H, 8.14; N, 5.95. Found: C, 76.49, H, 8.28; N, 6.10.

**(2*R*,3*S*)-2-(Tert-butyldimethylsilyloxy)-3-(dibenzylamino)-4-phenylbutanenitrile (57).**

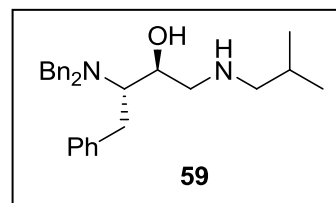
Title compound **57** was prepared starting from the protected cyanohydrin **56** as per the procedure outlined for the preparation of compound **54**. Yield: 78%; colourless syrup;  $[\alpha]_D^{20} = +6.79$  (*c* 1.2, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>): 3020, 2955, 1602, 1495, 1454, 1362, 1216 cm<sup>-1</sup>;



<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  -0.06 (s, 3H), 0.00 (s, 3H), 0.66 (t, *J* = 6.8, 7.2 Hz, 6H), 0.84 (s, 9H), 1.29-1.36 (m, 1H), 1.87-2.10 (m, 2H), 2.45-2.54 (m, 1H), 2.91-3.01 (m, 3H), 3.07-3.15 (m, 1H), 3.39 (d, *J* = 13.4 Hz, 2H), 3.66-3.74 (m, 1H), 4.11 (d, *J* = 13.4 Hz, 2H), 7.18-7.28 (m, 15H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  -4.6, -3.9, 18.2, 20.6, 20.7, 26.1, 28.5, 30.8, 52.7, 55.9, 57.3, 59.9, 73.2, 125.9, 126.8, 128.2, 128.4, 129.2, 129.4, 140.8, 141.2; (ESI):  $m/z$  531.8 (M+H)<sup>+</sup>, 553.8 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>34</sub>H<sub>50</sub>N<sub>2</sub>OSi: C, 76.93; H, 9.49; N, 5.28. Found: C, 76.85; H, 9.54; N, 5.24.

**(2*S*,3*S*)-3-(Dibenzylamino)-1-(isobutylamino)-4-phenylbutan-2-ol (59).**

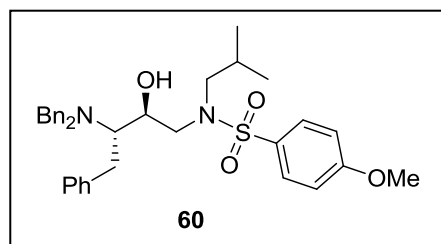
Title compound **59** was prepared starting from the compound **57** as per the procedure outlined for the preparation of compound **43**. Yield: 81%; colourless syrup;  $[\alpha]_{\text{D}}^{20} = +8.88$  (*c* 0.90, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>): 3683,



3338, 3019, 2958, 1602, 1521, 1495, 1454, 1216 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  0.82 (d, *J* = 6.7 Hz, 6H), 1.56-1.62 (m, 1H), 2.12-2.46 (m, 4H), 2.76-3.12 (m, 3H), 3.40 (d, *J* = 13.4 Hz, 2H), 3.64-3.68 (m, 1H), 3.99 (d, *J* = 13.4 Hz, 2H), 7.14-7.34 (m, 15H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  20.6, 28.5, 31.8, 53.4, 54.4, 57.9, 61.8, 69.7, 126.2, 127.2, 128.4, 128.5, 129.1, 129.4, 139.9, 140.4; (ESI): *m/z* 417.5 (M+H)<sup>+</sup>, 439.5 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>28</sub>H<sub>36</sub>N<sub>2</sub>O: C, 80.73; H, 8.71; N, 6.72. Found: C, 80.81; H, 8.65; N, 6.81.

***N*-((2*S*,3*S*)-3-(Dibenzylamino)-2-hydroxy-4-phenylbutyl)-*N*-isobutyl-4-methoxybenzenesulfonamide (60).**

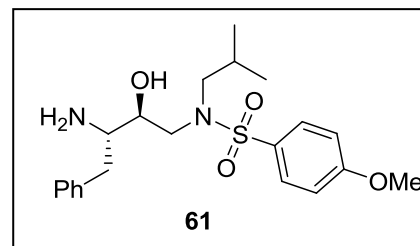
Title compound **60** was prepared starting from the compound **59** as per the procedure outlined for the preparation of compound **55**. Yield: 83%; colourless syrup;  $[\alpha]_{\text{D}}^{20} = +5.0$  (*c* 1.0, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>): 3515, 3019, 2966, 1598, 1496, 1454,



1259, 1216 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  0.68 (d, *J* = 6.2 Hz, 6H), 1.40-1.58 (m, 1H), 2.53-2.69 (m, 3H), 2.80-2.91 (m, 1H), 2.96-3.15 (m, 2H), 3.21-3.33 (m, 1H), 3.41 (d, *J* = 13.4 Hz, 2H), 3.60-3.66 (m, 1H), 3.74 (bs, 1H), 3.87 (s, 3H), 4.09 (d, *J* = 13.4 Hz, 2H), 6.93 (d, *J* = 9.0 Hz, 2H), 7.21-7.32 (m, 15H), 7.64 (d, *J* = 9.0 Hz, 2H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  19.8, 20.0, 27.0, 30.8, 54.8, 55.0, 55.6, 57.9, 61.9, 70.0, 114.1, 126.1, 127.1, 128.3, 128.6, 129.1, 129.3, 129.4, 131.0, 139.7, 140.2, 162.7; (ESI): *m/z* 587.5 (M+H)<sup>+</sup>, 589.5 (M+Na)<sup>+</sup>, 609.5 (M+K)<sup>+</sup>; Anal. Calcd for C<sub>35</sub>H<sub>42</sub>N<sub>2</sub>O<sub>4</sub>S: C, 71.64; H, 7.21; N, 4.77. Found: C, 71.76; H, 7.28; N, 4.86.

***N*-((2*S*,3*S*)-3-Amino-2-hydroxy-4-phenylbutyl)-*N*-isobutyl-4-methoxybenzenesulfonamide (61).**

Title compound **61** was prepared starting from the compound **60** as per the procedure outlined for the preparation of **42**. Yield: 80%; colourless solid;  $[\alpha]_D^{20} = -3.84$  ( $c$  1.0,  $\text{CHCl}_3$ ); mp 148-149 °C ; IR ( $\text{CHCl}_3$ ): 3396, 3018, 2969, 1597, 1496, 1334,



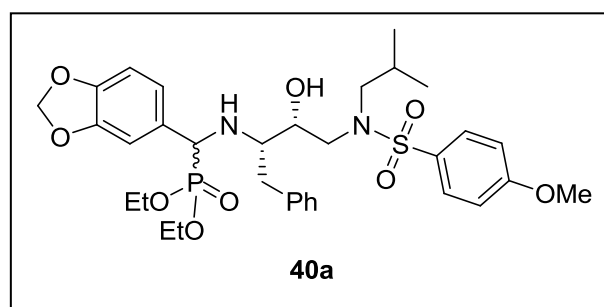
1261, 1217, 1155  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.78 (d,  $J = 6.7$  Hz, 3H), 0.80 (d,  $J = 6.6$  Hz, 3H), 1.61-1.75 (m, 1H), 2.37 (bs, 3H), 2.67-2.74 (m, 2H), 2.84-2.96 (m, 3H), 3.25-3.32 (m, 2H), 3.57-3.64 (m, 1H), 3.85 (s, 3H), 6.96 (d,  $J = 8.8$  Hz, 2H), 7.20-7.30 (m, 5H), 7.72 (d,  $J = 8.8$  Hz, 2H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  19.9, 20.1, 27.4, 40.6, 52.7, 53.5, 55.6, 58.7, 70.1, 114.3, 126.5, 128.6, 129.4, 130.4, 138.6, 162.9; (ESI):  $m/z$  407.4 ( $\text{M}+\text{H}$ ) $^+$ , 429.4 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{21}\text{H}_{30}\text{N}_2\text{O}_4\text{S}$ : C, 62.04; H, 7.44; N, 6.89. Found: C, 62.15, H, 7.52; N, 6.73.

### General experimental procedure for the synthesis of $\alpha$ -aminophosphonate:

Aldehyde (1 mmol), amine **42** or **61** (1 mmol), diethylphosphite (1 mmol) and Amberlite IR 120 (100 mg) were taken in a Pyrex test tube and exposed to microwave irradiation (Kenstar Model No. OM-9918C; 2450 MHz, 2350 W) for appropriate time (see Table 1). After completion of the reaction (TLC), the reaction mixture was cooled and DCM (25 mL) was added. The catalyst was filtered out from the reaction mixture and filtrate was concentrated under vacuum. The residue was chromatographed over silica gel column (100-200 mesh) and eluted with petroleum ether: ethyl acetate (2:3 to 3:2) to afford the corresponding pure  $\alpha$ -aminophosphonates.

**Diethyl (S)-benzo[d][1,3]dioxol-5-yl((2S,3R)-3-hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylamino)methylphosphonate (40a)** (1:5 diastereomeric mixture, only peaks corresponding to the major isomer are given).

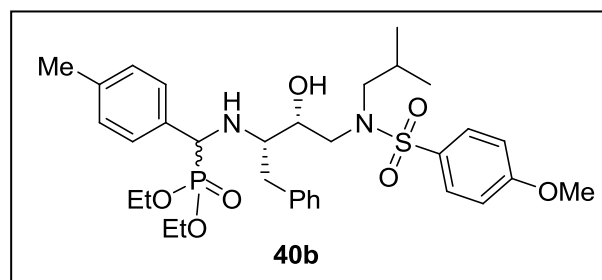
Yield: 53%; colourless syrup; IR ( $\text{CHCl}_3$ ): 3340, 3022, 2400, 1598, 1494, 1440, 1300, 1210, 1150  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.83 (d,  $J = 6.5$  Hz, 3H), 0.85 (d,  $J = 6.8$  Hz, 3H), 1.14 (t,  $^3J_{\text{PH}} = 7.0$  Hz, 3H),



1.24 (t,  $^3J_{\text{PH}} = 7.3$  Hz, 3H), 1.69-1.76 (m, 1H), 2.70-2.75 (m, 1H), 2.81-2.82 (m, 2H), 2.89-2.91 (m, 1H), 2.93-2.97 (m, 1H), 3.07-3.22 (m 2H), 3.51-3.55 (m, 1H), 3.71-3.98 (m, 4H), 3.89 (s, 3H), 3.96 (d,  $^2J_{\text{PH}} = 19.6$  Hz, 1H), 5.97 (s, 2H), 6.73-6.74 (m, 2H), 6.85 (m, 1H), 6.99 (d,  $J = 9.0$  Hz, 2H), 7.23-7.34 (m, 5H), 7.68 (d,  $J = 9.0$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.3 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 16.4 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 19.8, 20.1, 27.2, 35.1, 52.8, 55.6, 58.3 (d,  $^1J_{\text{PC}} = 152.6$  Hz), 58.3, 59.2 (d,  $^3J_{\text{PC}} = 13.9$  Hz), 62.5 (d,  $^2J_{\text{PC}} = 7.3$  Hz), 62.9 (d,  $^2J_{\text{PC}} = 7.3$  Hz), 70.6, 101.1, 108.1, 109.0 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 114.2, 122.6 (d,  $^3J_{\text{PC}} = 7.3$  Hz), 126.5, 128.5, 129.4, 129.6, 129.7, 130.4, 137.9, 147.4, 147.8, 162.8;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  23.57; (ESI):  $m/z$  699.5 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{33}\text{H}_{45}\text{N}_2\text{O}_9\text{PS}$ : C, 58.57; H, 6.70; N, 4.14. Found: C, 58.69; H, 6.81; N, 4.21.

**Diethyl ((2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylamino)(*p*-tolyl)methylphosphonate (40b)** (1:4 diastereomeric mixture, only peaks corresponding to the major isomer are given).

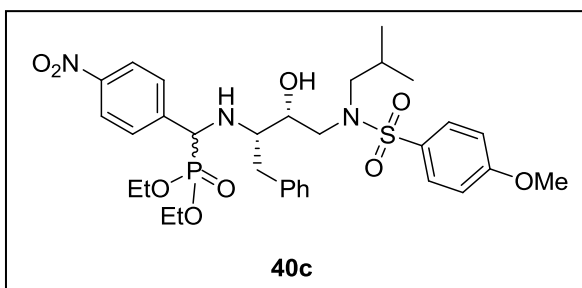
Yield: 40%; colourless syrup; IR ( $\text{CHCl}_3$ ): 3351, 3019, 1598, 1513, 1497, 1412, 1215, 1155, 1030  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.89 (d,  $J = 6.5$  Hz, 3H), 0.90 (d,  $J = 6.4$  Hz, 3H), 1.08 (t,  $^3J_{\text{PH}} = 7.0$  Hz, 3H),



1.27 (t,  $^3J_{\text{PH}} = 7.3$  Hz, 3H), 1.88-1.95 (m, 1H), 2.31 (s, 3H), 2.47-2.53 (m, 1H), 2.76-2.82 (m, 2H), 2.89-3.06 (m, 2H), 3.11-3.16 (m, 1H), 3.32-3.36 (m, 1H), 3.63-3.71 (m, 1H), 3.84-4.06 (m, 4H), 3.89 (s, 3H), 3.99 (d,  $^2J_{\text{PH}} = 23.1$  Hz, 1H), 6.64-6.67 (m, 2H), 6.93-7.06 (m, 6H), 7.25-7.28 (m, 3H), 7.75 (d,  $J = 8.9$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.2 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 16.4 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 20.0, 20.1, 27.1, 34.8, 51.8, 55.6, 57.2 (d,  $^1J_{\text{PC}} = 158.5$  Hz), 57.9, 58.4 (d,  $^3J_{\text{PC}} = 15.4$  Hz), 62.4 (d,  $^2J_{\text{PC}} = 7.3$  Hz), 62.9 (d,  $^2J_{\text{PC}} = 6.6$  Hz), 69.7, 114.2, 126.6, 128.0 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 128.6, 129.0, 129.4, 129.4, 131.0, 131.9, 137.4, 137.9, 162.8;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  23.55; (ESI):  $m/z$  647.9 ( $\text{M}+\text{H}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{33}\text{H}_{47}\text{N}_2\text{O}_7\text{PS}$ : C, 61.28; H, 7.32; N, 4.33. Found: C, 61.37; H, 7.38; N, 4.26.

**Diethyl ((2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylamino)(4-nitrophenyl)methylphosphonate (40c)** (1:4 diastereomeric mixture, only peaks corresponding to the major isomer are given).

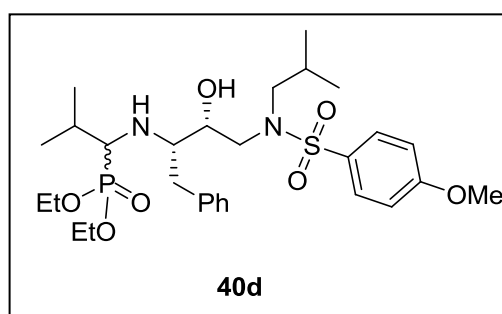
Yield: 38%; colourless syrup; IR (CHCl<sub>3</sub>): 3350, 3019, 2923, 1604, 1523, 1495, 1456, 1397, 1348, 1217, 1156, 1081, 1030 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.82 (d, *J* = 6.6 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H),



1.14(t, <sup>3</sup>*J*<sub>PH</sub> = 7.2 Hz, 3H), 1.22 (t, <sup>3</sup>*J*<sub>PH</sub> = 7.2 Hz, 3H), 1.65-1.70 (m, 1H), 2.72-2.78 (m, 2H), 2.82-2.83 (m, 1H), 2.94-2.97 (m, 2H), 3.06-3.13 (m, 2H), 3.47-3.65 (m, 1H), 3.77-3.98 (m, 4H), 3.91 (s, 3H), 4.22 (d, <sup>2</sup>*J*<sub>PH</sub> = 21.7 Hz, 1H), 6.98 (d, *J* = 8.8 Hz, 2H); 7.21-7.34 (m, 5H), 7.46- 7.48 (m, 2H), 7.61 (d, *J* = 8.8 Hz, 2H), 8.18 (d, *J* = 8.3 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 16.3 (d, <sup>3</sup>*J*<sub>PC</sub> = 5.1 Hz), 16.4 (d, <sup>3</sup>*J*<sub>PC</sub> = 5.1 Hz), 19.8, 20.1, 27.2, 36.0, 52.4, 55.6, 58.3 (d, <sup>1</sup>*J*<sub>PC</sub> = 146.7 Hz), 58.6, 59.9 (d, <sup>3</sup>*J*<sub>PC</sub> = 11.7 Hz), 62.9 (d, <sup>2</sup>*J*<sub>PC</sub> = 6.6 Hz), 63.1 (d, <sup>2</sup>*J*<sub>PC</sub> = 6.6 Hz), 71.4, 114.3, 123.4, 126.8, 128.7, 129.4, 129.4, 129.6, 129.9, 137.8, 144.5, 147.5, 163.0; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 21.91; (ESI): *m/z* 700.6 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>32</sub>H<sub>44</sub>N<sub>3</sub>O<sub>9</sub>PS: C, 56.71; H, 6.54; N, 6.20. Found: C, 56.83; H, 6.62; N, 6.36.

**Diethyl 1-((2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylamino)-2-methylpropylphosphonate (40d)** (1:4 diastereomeric mixture, only peaks corresponding to the major isomer are given).

Yield: 76%; colourless syrup; IR (CHCl<sub>3</sub>): 3349, 3019, 2966, 2873, 1598, 1579, 1497, 1465, 1391, 1337, 1260, 1216, 1154, 1093, 1028 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 0.59 (d, *J* = 6.8 Hz, 3H), 0.60 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.5 Hz, 3H), 0.95 (d, *J* =

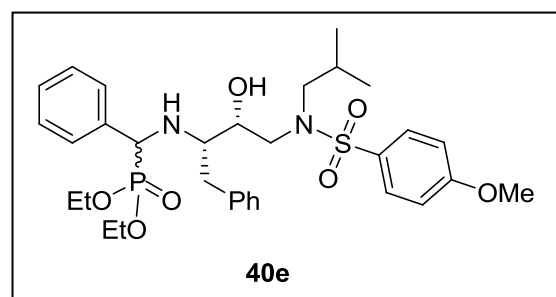


6.5 Hz, 3H), 1.28-1.33 (m, 6H), 1.77-1.83 (m, 1H), 2.03-2.10 (m, 1H), 2.53-2.61 (m, 2H), 2.82-3.04 (m, 4H), 3.17-3.23 (m, 1H), 3.47-3.63 (m, 1H), 3.82-3.84 (m, 1H), 3.86 (s, 3H), 3.93-4.18 (m, 4H), 6.98 (d, *J* = 8.8 Hz, 2H), 7.23-7.34 (m, 5H), 7.78 (d, *J* = 8.8 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 16.4 (d, <sup>3</sup>*J*<sub>PC</sub> = 5.9 Hz), 16.6 (d, <sup>3</sup>*J*<sub>PC</sub> = 5.9 Hz), 17.3 (d, <sup>3</sup>*J*<sub>PC</sub> = 2.9 Hz), 20.0, 20.1, 26.9, 29.5 (d, <sup>2</sup>*J*<sub>PC</sub> = 3.7 Hz), 34.4, 51.1,

55.5, 57.2, 57.7 (d,  $^1J_{PC} = 162.1$  Hz), 61.6 (d,  $^2J_{PC} = 8.1$  Hz), 62.6 (d,  $^2J_{PC} = 10.3$  Hz), 63.2 (d,  $^2J_{PC} = 7.3$  Hz), 69.4, 114.2, 126.7, 128.7, 129.3, 129.4, 131.6, 138.6, 162.7;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  27.36; (ESI):  $m/z$  621.5 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{29}\text{H}_{47}\text{N}_3\text{O}_7\text{PS}$ : C, 58.17; H, 7.91; N, 4.68. Found: C, 58.28; H, 7.98; N, 4.77.

**Diethyl ((2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylamino)(phenyl)methylphosphonate (40e)** (1:3 diastereomeric mixture, only peaks corresponding to the major isomer are given).

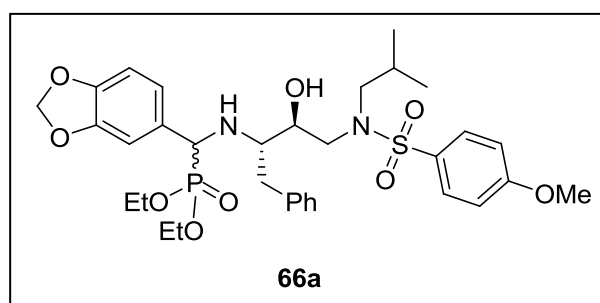
Yield: 40%; colourless syrup; IR ( $\text{CHCl}_3$ ): 3430, 3019, 1598, 1497, 1335, 1260, 1215, 1154  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.80 (d,  $J = 6.6$  Hz, 3H), 0.82 (d,  $J = 6.4$  Hz, 3H), 1.07 (t,  $^3J_{\text{PH}} = 7.1$  Hz, 3H), 1.21 (t,  $^3J_{\text{PH}} = 7.1$



Hz, 3H), 1.61-1.76 (m, 1H), 2.66-2.76 (m, 1H), 2.78-2.86 (m, 2H), 2.90-2.91 (m, 1H), 2.94-2.97 (m, 1H), 3.05-3.23 (m, 2H), 3.48-3.54 (m, 1H), 3.63-4.05 (m, 4H), 3.88 (s, 3H), 4.05 (d,  $^2J_{\text{PH}} = 20.1$  Hz, 1H), 6.95 (d,  $J = 9.0$  Hz, 2H), 7.20-7.30 (m, 10H), 7.64 (d,  $J = 8.8$  Hz, 2H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.2 (d,  $^3J_{PC} = 5.9$  Hz), 16.4 (d,  $^3J_{PC} = 5.9$  Hz), 20.0, 20.1, 27.2, 34.9, 51.9, 55.6, 57.7 (d,  $^1J_{PC} = 158.1$  Hz), 57.9, 58.5 (d,  $^3J_{PC} = 15.7$  Hz), 62.6 (d,  $^2J_{PC} = 7.3$  Hz), 63.0 (d,  $^2J_{PC} = 7.3$  Hz), 69.9, 114.2, 126.6, 127.7, 127.8, 128.1, 128.3, 128.5, 128.7, 129.3, 129.4, 130.9, 135.2, 137.9, 162.8;  $^{31}\text{P}$  NMR (202 MHz,  $\text{CDCl}_3$ ):  $\delta$  23.70; (ESI):  $m/z$  633.3 ( $\text{M}+\text{H}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{32}\text{H}_{45}\text{N}_2\text{O}_7\text{PS}$ : C, 60.74; H, 7.17; N, 4.43. Found: C, 60.79; H, 7.24; N, 4.35.

**Diethyl benzo[d][1,3]dioxol-5-yl((2*S*,3*S*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylamino)methylphosphonate (66a)** (2:3 diastereomeric mixture, only peaks corresponding to the major isomer are given).

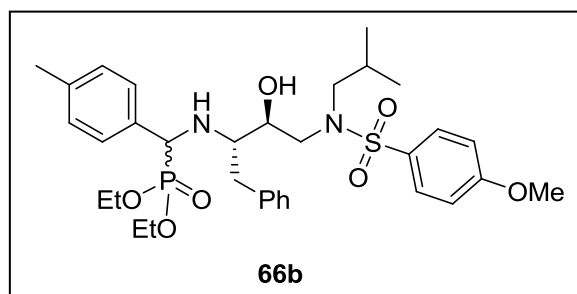
Yield: 52%; colourless syrup; IR ( $\text{CHCl}_3$ ): 3346, 3019, 1597, 1497, 1442, 1303, 1216, 1154  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.71 (d,  $J = 6.8$  Hz, 3H), 0.74 (d,  $J = 6.8$  Hz, 3H), 1.09-1.32 (m, 6H), 1.45-1.78



(m, 1H), 2.39-2.65 (m, 2H), 2.73-2.85 (m, 3H), 2.89-3.06 (m, 1H), 3.35-3.41 (m, 1H), 3.56-3.77 (m, 1H), 3.89 (s, 3H), 3.93-4.15 (m, 5H), 5.97 (s, 2H), 6.51-7.28 (m, 10H), 7.69 (d,  $J = 8.8$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.4 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 19.7, 20.0, 27.2, 36.1, 53.9, 55.6, 57.8 (d,  $^1J_{\text{PC}} = 159.9$  Hz), 58.2, 58.5 (d,  $^3J_{\text{PC}} = 7.3$  Hz), 62.9 (d,  $^2J_{\text{PC}} = 7.3$  Hz), 62.6 (d,  $^2J_{\text{PC}} = 7.3$  Hz), 69.5, 101.1, 108.0, 109.2 (d,  $^3J_{\text{PC}} = 5.5$  Hz), 114.2, 122.2 (d,  $^3J_{\text{PC}} = 8.8$  Hz), 126.5, 128.5, 129.4, 129.5, 129.7, 130.4, 138.0, 147.4, 147.7, 162.8;  $^{31}\text{P}$  NMR (202 MHz,  $\text{CDCl}_3$ ):  $\delta$  23.70; (ESI):  $m/z$  677.6 ( $\text{M}+\text{H}$ ) $^+$ , 699.6 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{33}\text{H}_{45}\text{N}_2\text{O}_9\text{PS}$ : C, 58.57; H, 6.70; N, 4.14. Found: C, 58.64; H, 6.79; N, 4.27.

**Diethyl ((2*S*,3*S*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylamino)(*p*-tolyl)methylphosphonate (66b)** (3:2 diastereomeric mixture, only peaks corresponding to the major isomer are given).

Yield: 78%; colourless syrup; IR ( $\text{CHCl}_3$ ): 3436, 3019, 1598, 1497, 1335, 1260, 1215, 1154  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.67 (d,  $J = 5.7$  Hz, 3H), 0.71 (d,  $J = 5.7$  Hz, 3H), 1.16-1.32 (m, 6H), 1.40-1.75 (m, 1H),

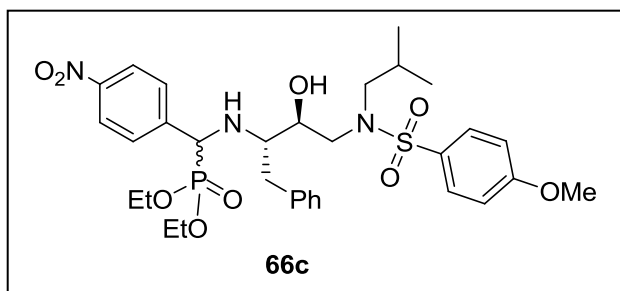


2.33 (s, 3H), 2.39-2.70 (m 2H), 2.76-2.87 (m, 3H), 2.94-3.11 (m, 1H), 3.28-3.39 (m, 1H), 3.50-3.70 (m, 1H), 3.88 (s, 3H), 3.83-4.20 (m, 5H), 6.91-7.26 (m, 11H), 7.67 (d,  $J = 9.1$  Hz, 2H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.5 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 19.7, 20.1, 21.2, 27.1, 36.1, 53.9, 55.6, 57.8 (d,  $^1J_{\text{PC}} = 151.8$  Hz), 58.1, 58.5, 58.8, 62.6 (d,  $^2J_{\text{PC}} = 7.3$  Hz), 63.0 (d,  $^2J_{\text{PC}} = 7.3$  Hz), 69.4, 114.2, 126.3, 128.2, 128.5, 128.9, 129.0, 129.2, 130.5, 132.6, 137.7, 138.8, 162.8;  $^{31}\text{P}$  NMR (202 MHz,  $\text{CDCl}_3$ ):  $\delta$  23.90; (ESI):  $m/z$  669.6 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{33}\text{H}_{47}\text{N}_2\text{O}_7\text{PS}$ : C, 61.28; H, 7.32; N, 4.33. Found: C, 61.39; H, 7.47; N, 4.26.

**Diethyl ((2*S*,3*S*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylamino)(4-nitrophenyl)methylphosphonate (66c)** (3:2 diastereomeric mixture, only peaks corresponding to the major isomer are given).

Yield: 56%; colourless syrup; IR ( $\text{CHCl}_3$ ): 3463, 3019, 1597, 1523, 1348, 1260, 1216, 1155  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.69 (m, 6H), 1.05-1.35 (m, 6H), 1.54-1.85 (m, 1H), 2.26-2.69 (m, 2H), 2.75-2.88 (m, 3H), 2.91-3.03 (m, 1H), 3.26-3.44 (m, 1H),

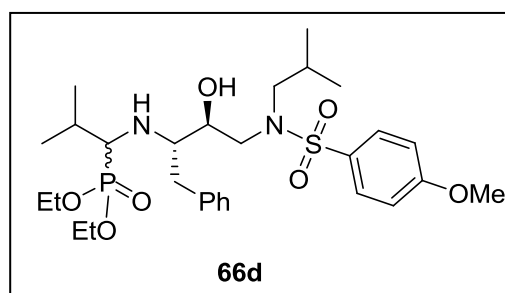
3.57-3.83 (m, 1H), 3.89 (s, 3H),  
 3.93-4.25 (m, 4H), 4.30 (d,  $^2J_{\text{PH}} =$   
 21.3 Hz, 1H), 6.92-7.29 (m, 8H),  
 7.50-7.56 (m, 1H), 7.67 (d,  $J = 8.9$   
 Hz, 2H), 8.17 (d,  $J = 8.2$  Hz, 2H);  
 $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.3



(d,  $^3J_{\text{PC}} = 5.9$  Hz), 16.5 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 19.7, 20.0, 27.3, 36.5, 53.9, 55.7, 57.8 (d,  $^1J_{\text{PC}} = 148.6$  Hz), 58.5, 58.8, 63.0 (d,  $^2J_{\text{PC}} = 6.9$  Hz), 63.2 (d,  $^2J_{\text{PC}} = 6.9$  Hz), 69.5, 114.3, 123.4, 126.5, 128.6, 129.2, 129.4, 129.8, 130.0, 138.4, 143.9, 147.6, 163.0;  $^{31}\text{P}$  NMR (202 MHz,  $\text{CDCl}_3$ ):  $\delta$  21.85; (ESI):  $m/z$  700.6 ( $\text{M}+\text{Na}^+$ ); Anal. Calcd for  $\text{C}_{32}\text{H}_{44}\text{N}_3\text{O}_9\text{PS}$ : C, 56.71; H, 6.54; N, 6.20. Found: C, 56.78; H, 6.63; N, 6.32.

**Diethyl 1-((2*S*, 3*S*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylamino)-2-methylpropylphosphonate (66d)** (1:1 diastereomeric mixture, only peaks corresponding to the major isomer are given).

Yield: 70%; colourless syrup; IR ( $\text{CHCl}_3$ ):  
 3352, 3019, 1598, 1497, 1335, 1259, 1216,  
 1154  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$   
 0.63 (d,  $J = 6.6$  Hz, 3H), 0.78 (d,  $J = 6.6$  Hz,  
 3H), 0.81 (d,  $J = 6.8$  Hz, 3H), 1.04 (d,  $^3J_{\text{PH}} =$   
 6.8 Hz, 3H), 1.30-1.40 (m, 6H), 1.69-1.74

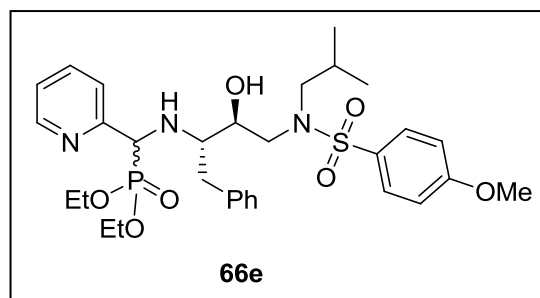


(m, 1H), 2.07-2.23 (m, 1H), 2.68-2.71 (m, 1H), 2.82-2.89 (m, 2H), 2.94-3.07 (m, 2H),  
 3.12-3.22 (m, 2H), 3.32-3.35 (m, 1H), 3.54-3.59 (m, 1H), 3.88 (s, 3H), 4.09-4.20 (m,  
 4H), 6.96 (d,  $J = 8.8$  Hz, 2H), 7.22-7.34 (m, 5H), 7.67 (d,  $J = 8.8$  Hz, 2H);  $^{13}\text{C}$  NMR  
 (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.5 (d,  $^3J_{\text{PC}} = 5.5$  Hz), 17.7 (d,  $^3J_{\text{PC}} = 2.2$  Hz), 18.3 (d,  $^3J_{\text{PC}} =$   
 2.5 Hz), 19.9, 27.2, 29.4 (d,  $^2J_{\text{PC}} = 3.6$  Hz), 38.0, 53.4, 55.6, 57.9, 58.4 (d,  $^1J_{\text{PC}} =$   
 158.9 Hz), 60.5, 61.5 (d,  $^2J_{\text{PC}} = 7.3$  Hz), 61.5 (d,  $^2J_{\text{PC}} = 7.3$  Hz), 67.8, 114.1, 126.4,  
 128.5, 129.3, 129.5, 130.7, 138.6, 162.7;  $^{31}\text{P}$  NMR (202 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.52;  
 (ESI):  $m/z$  621.5 ( $\text{M}+\text{Na}^+$ ); Anal. Calcd for  $\text{C}_{29}\text{H}_{47}\text{N}_3\text{O}_7\text{PS}$ : C, 58.17; H, 7.91; N,  
 4.68. Found: C, 58.31; H, 8.09; N, 4.59.

**Diethyl ((2*S*,3*S*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylamino)(pyridin-2-yl)methylphosphonate (66e)** (1:1 diastereomeric mixture, only peaks corresponding to the major isomer are given).



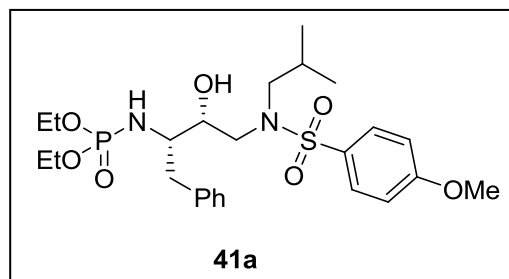
Yield: 63%; colourless syrup; IR (CHCl<sub>3</sub>): 3379, 3019, 2931, 1597, 1497, 1468, 1434, 1259, 1215, 1154 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 0.69 (d, *J* = 6.8 Hz, 3H), 0.78 (d, *J* = 6.8 Hz, 3H), 1.16 (t, <sup>3</sup>*J*<sub>PH</sub> = 7.0 Hz, 3H), 1.28 (t, <sup>3</sup>*J*<sub>PH</sub> = 7.0 Hz, 3H), 1.49-



1.68 (m, 1H), 2.51-2.57 (m, 1H), 2.71-2.91 (m, 4H), 3.10-3.15 (m, 1H), 3.40-3.45 (m, 1H), 3.59-3.64 (m, 1H), 3.89 (s, 3H), 3.78-4.11 (m, 4H), 4.45 (d, <sup>2</sup>*J*<sub>PH</sub> = 22.1 Hz, 1H), 6.98-7.00 (m, 2H), 7.10-7.23 (m, 5H), 7.44-7.54 (m, 3H), 7.77 (d, *J* = 9.0 Hz, 2H), 8.47 (m, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 16.4 (d, <sup>3</sup>*J*<sub>PC</sub> = 5.9 Hz), 19.9, 20.1, 27.2, 38.7, 53.7, 55.6, 58.1, 61.8 (d, <sup>1</sup>*J*<sub>PC</sub> = 149.6 Hz), 60.9, 62.7 (d, <sup>2</sup>*J*<sub>PC</sub> = 7.3 Hz), 62.8 (d, <sup>2</sup>*J*<sub>PC</sub> = 7.3 Hz), 70.1, 114.2, 122.5, 123.6, 126.2, 128.4, 129.3, 129.5, 130.7, 136.2, 138.3, 149.0, 155.7, 162.8; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 22.40; (ESI): *m/z* 656.5 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>31</sub>H<sub>44</sub>N<sub>3</sub>O<sub>7</sub>PS: C, 58.75; H, 7.00; N, 6.63. Found: C, 58.68; H, 7.12; N, 6.49.

**Diethyl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylphosphoramidate (41a)**

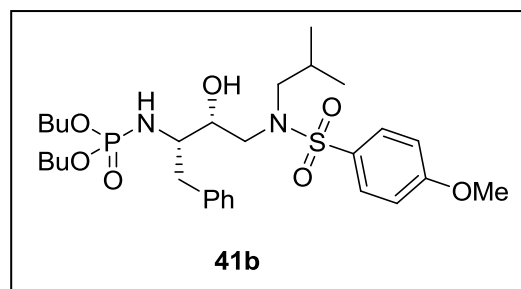
Yield: 76%; colourless syrup; [α]<sub>D</sub><sup>20</sup> = +10.82 (*c* 0.6, MeOH); IR (CHCl<sub>3</sub>): 3281, 3020, 1689, 1604, 1542, 1497, 1456, 1395, 1335, 1262, 1215, 1155, 1046 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 0.87 (d, *J* = 6.6



Hz, 3H), 0.88 (d, *J* = 6.6 Hz, 3H), 1.14 (t, <sup>3</sup>*J*<sub>PH</sub> = 7.1 Hz, 3H), 1.18 (t, <sup>3</sup>*J*<sub>PH</sub> = 7.1 Hz, 3H), 1.81-1.95 (m, 1H), 2.85-2.94 (m, 3H), 3.08-3.16 (m, 2H), 3.26-3.51 (m, 3H), 3.75-3.88 (m, 4H), 3.86 (s, 3H), 4.22-4.30 (m, 1H), 6.97 (d, *J* = 8.9 Hz, 2H); 7.23-7.31 (m, 5H), 7.73 (d, *J* = 8.9 Hz, 2H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ 16.1 (d, <sup>3</sup>*J*<sub>PC</sub> = 7.3 Hz), 16.2 (d, <sup>3</sup>*J*<sub>PC</sub> = 7.3 Hz), 20.0, 20.1, 27.1, 36.9 (d, <sup>3</sup>*J*<sub>PC</sub> = 6.2 Hz), 52.9, 55.6, 56.6, 58.3, 62.2 (d, <sup>2</sup>*J*<sub>PC</sub> = 5.8 Hz), 62.5 (d, <sup>2</sup>*J*<sub>PC</sub> = 5.8 Hz), 72.8 (d, <sup>3</sup>*J*<sub>PC</sub> = 3.3 Hz), 114.3, 126.4, 128.4, 129.4, 129.9, 130.4, 138.3, 162.9; <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>): δ 8.22; (ESI): *m/z* 543.6 (M+H)<sup>+</sup>, 565.5 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>25</sub>H<sub>39</sub>N<sub>2</sub>O<sub>7</sub>PS: C, 55.34; H, 7.24; N, 5.16. Found: C, 55.39; H, 7.35; N, 5.21.

**Dibutyl (2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-ylphosphoramidate (41b)**

Yield: 61%; colourless syrup;  $[\alpha]_D^{20} = +5.42$  (*c* 0.5, MeOH); IR (CHCl<sub>3</sub>): 3392, 3019, 2963, 2874, 1597, 1578, 1497, 1456, 1391, 1337, 1260, 1216, 1154, 1092, 1030 cm<sup>-1</sup> <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  0.78-0.85 (m, 12H), 1.15-1.46 (m, 8H), 1.72-1.86 (m, 1H),

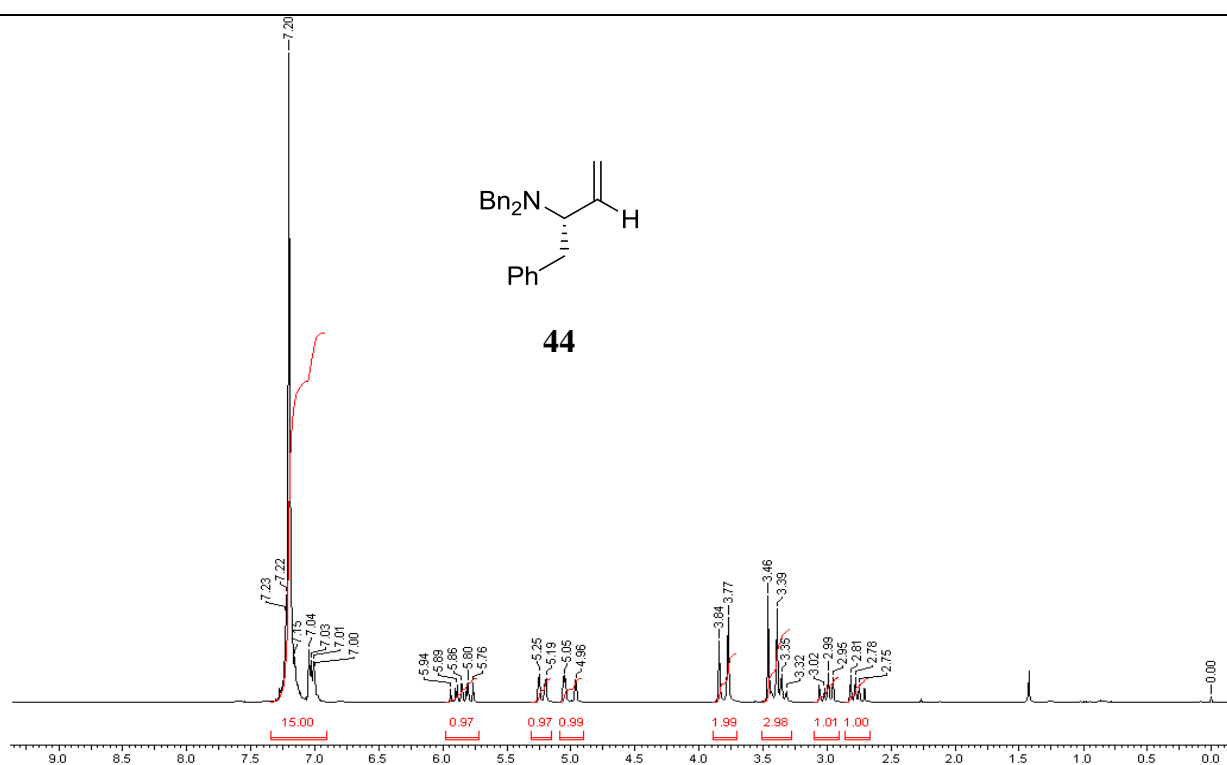
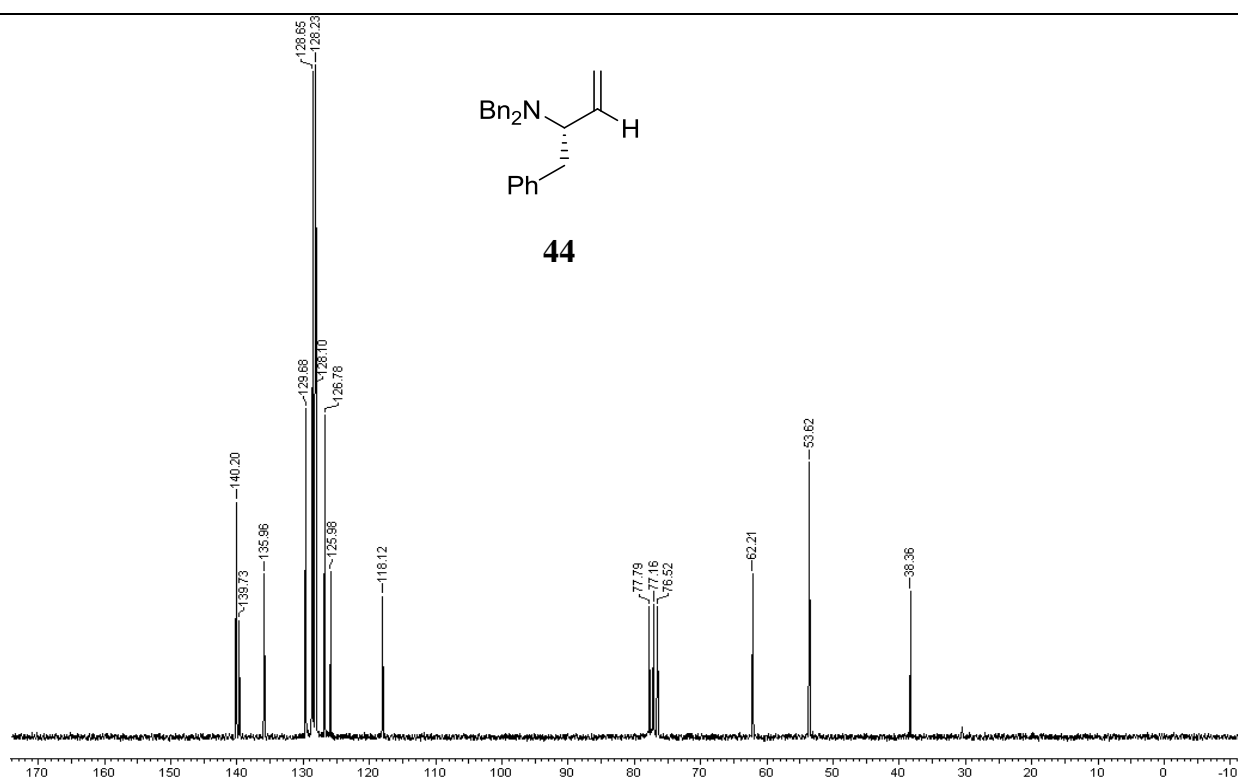


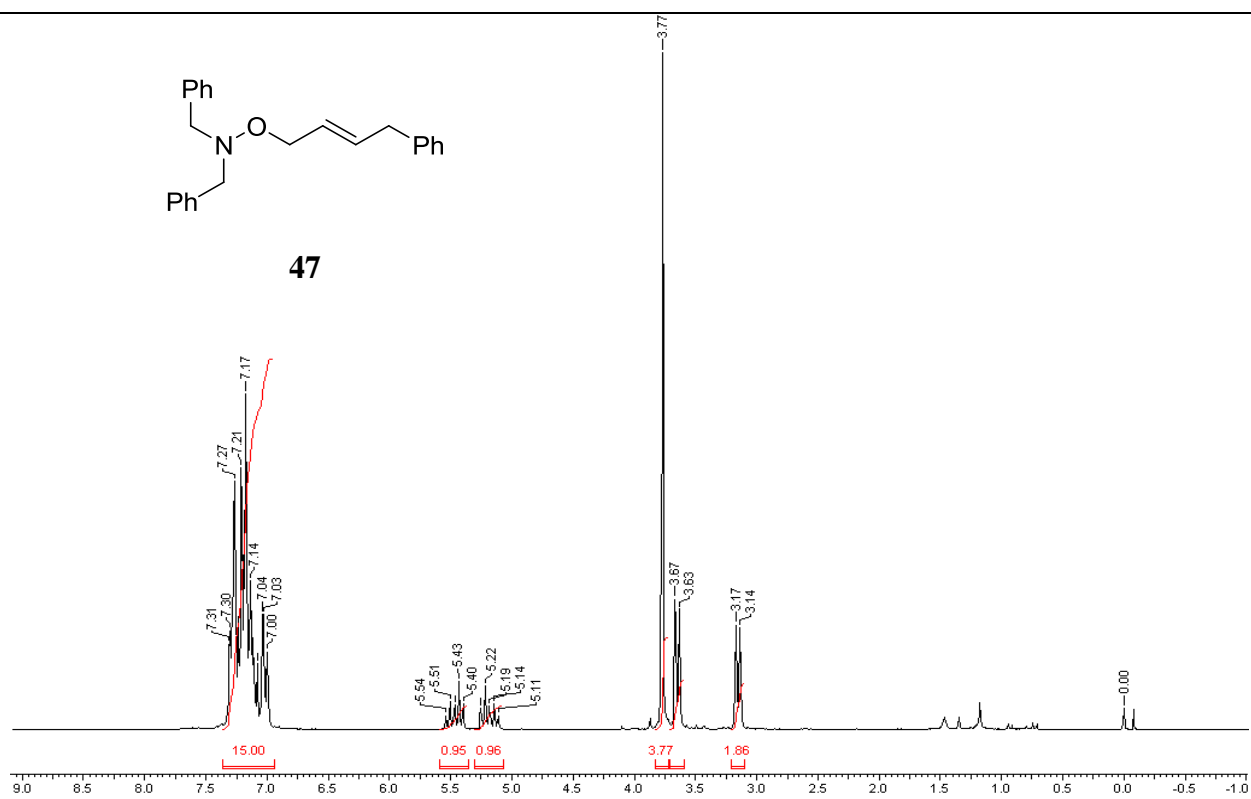
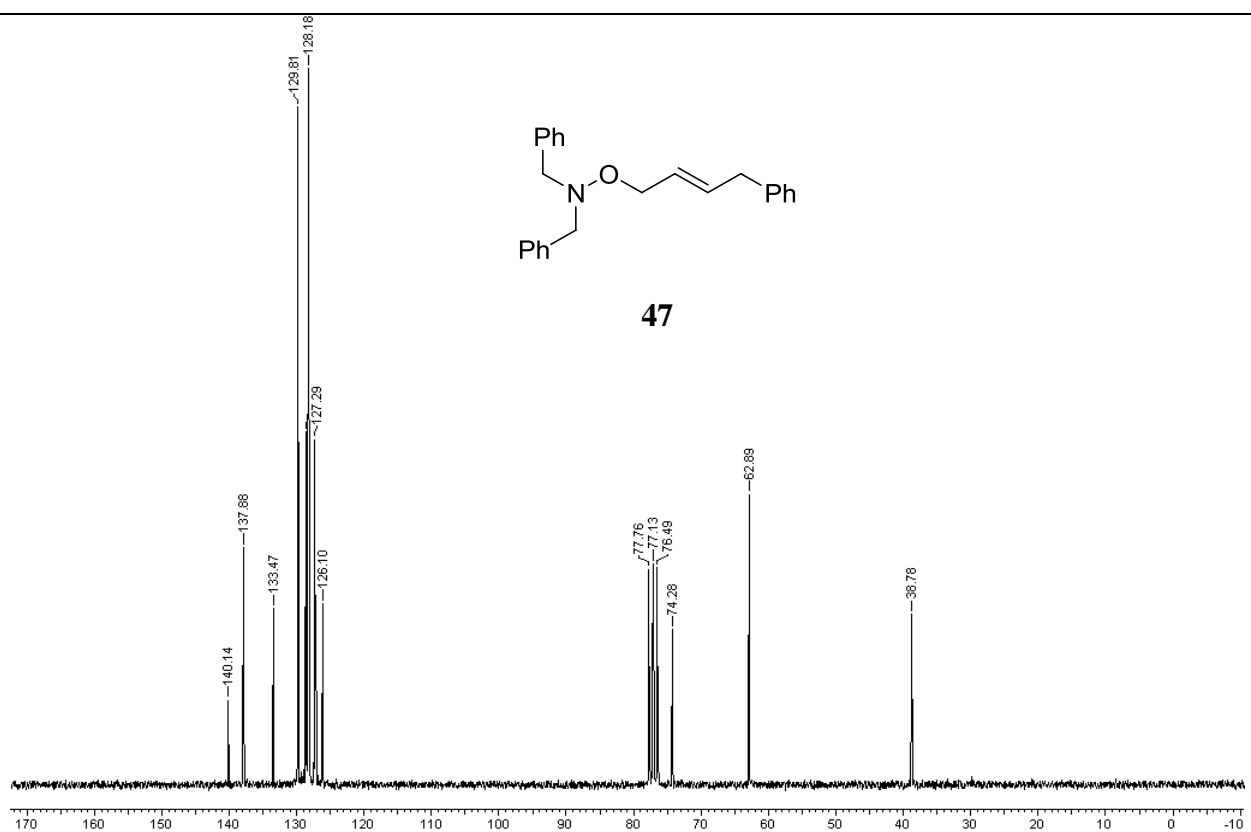
2.79-3.06 (m, 6H), 3.18-3.27 (m, 1H), 3.40-3.49 (m, 1H), 3.61-3.76 (m, 4H), 3.79 (s, 3H), 6.91 (d, *J* = 8.9 Hz, 2H); 7.13-7.23 (m, 5H), 7.65 (d, *J* = 8.9 Hz, 2H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  13.6, 18.7, 19.9, 20.2, 27.2, 32.2 (d, <sup>3</sup>*J*<sub>PC</sub> = 2.2 Hz), 32.4 (d, <sup>3</sup>*J*<sub>PC</sub> = 2.2 Hz), 37.2 (d, <sup>3</sup>*J*<sub>PC</sub> = 5.5 Hz), 53.1, 55.6, 56.4, 58.5, 66.1 (d, <sup>2</sup>*J*<sub>PC</sub> = 7.3 Hz), 66.2 (d, <sup>2</sup>*J*<sub>PC</sub> = 7.3 Hz), 72.7 (d, <sup>3</sup>*J*<sub>PC</sub> = 3.7 Hz), 114.3, 126.5, 128.4, 129.4, 129.9, 130.4, 138.0, 162.9; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>):  $\delta$  8.26; (ESI): *m/z* 621.5 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>29</sub>H<sub>47</sub>N<sub>2</sub>O<sub>7</sub>PS: C, 58.17; H, 7.91; N, 4.68. Found: C, 58.29; H, 8.05; N, 4.61.

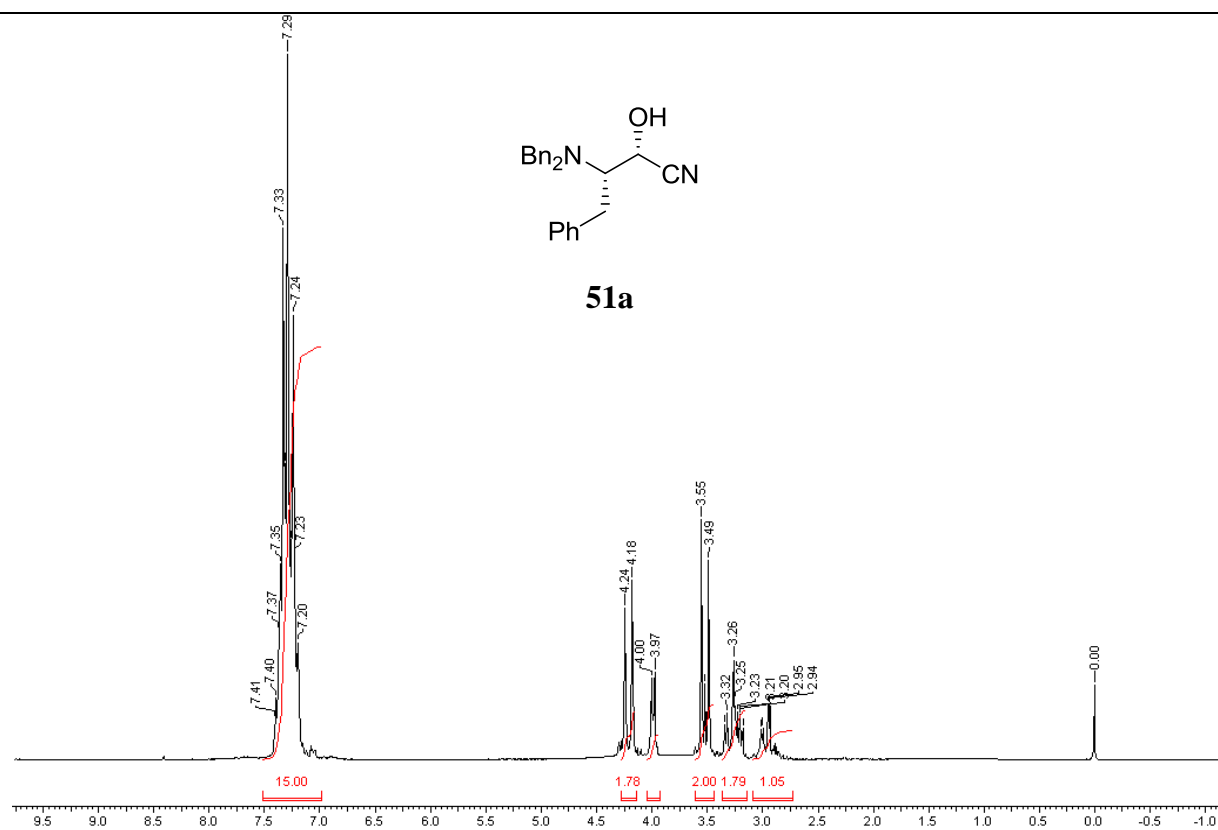
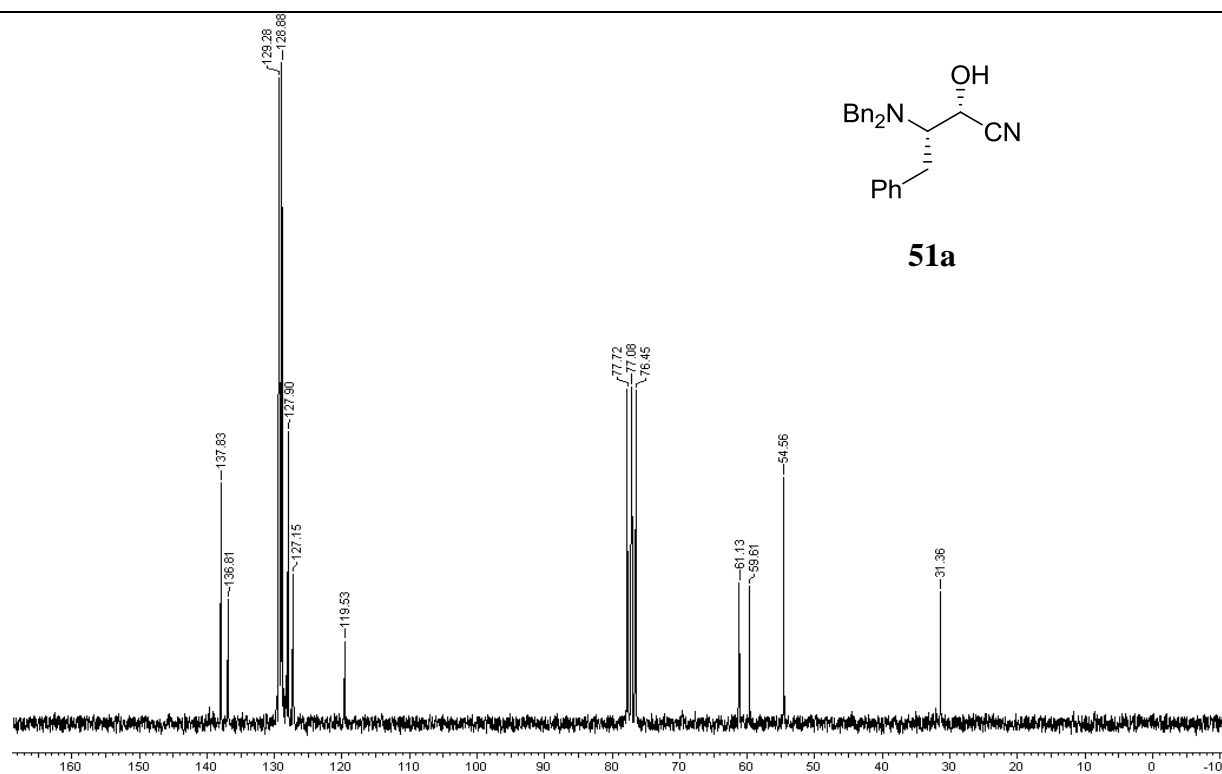
**Anti-HIV activity assays:**

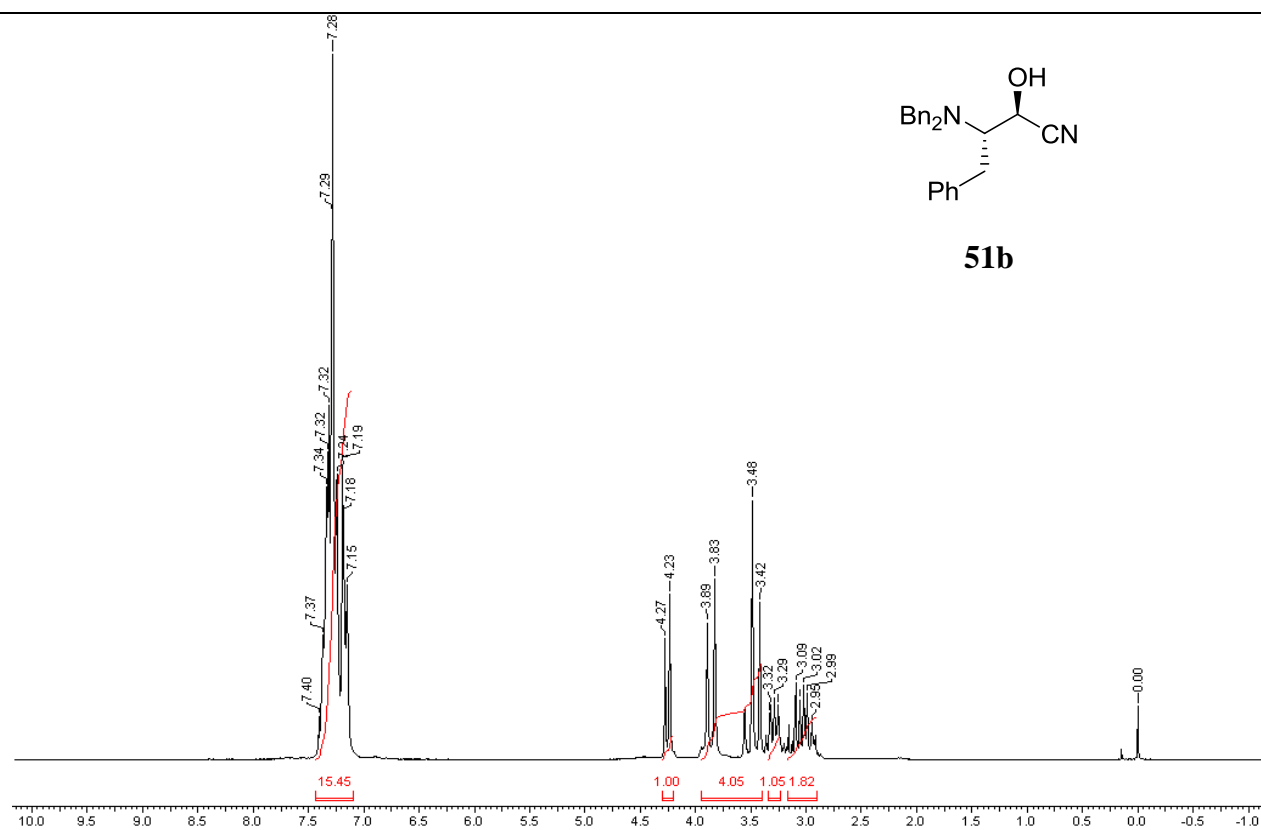
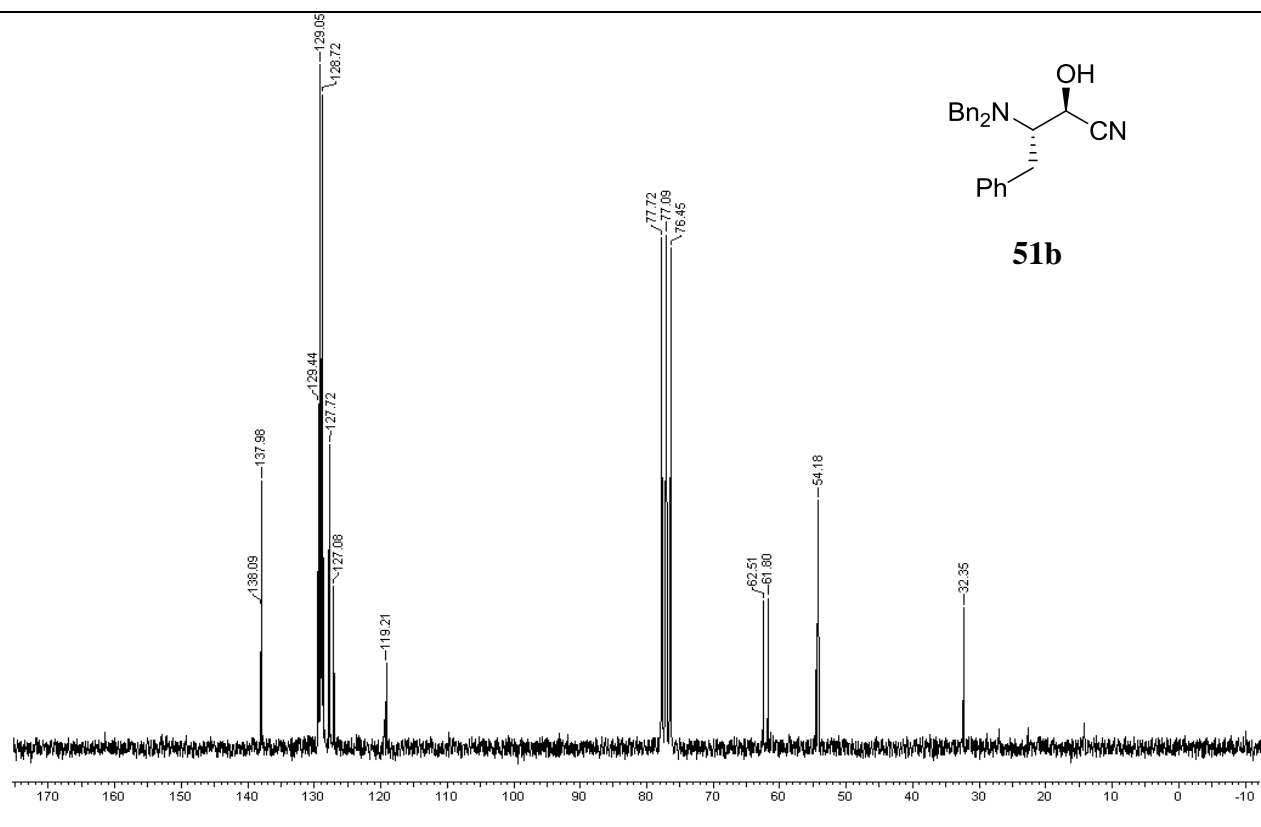
The anti-HIV activities and cytotoxicities of all the synthesized compounds were evaluated against wild-type HIV-1 (III<sub>B</sub>), HIV-2 (ROD) and double mutant (RES056) strains in MT-4 cells using the 3-(4,5-dimethylthiazoldimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.<sup>47</sup> Briefly, virus stocks were titrated in MT-4 cells and expressed as 50% cell culture infective dose (CCID<sub>50</sub>). MT-4 cells were suspended in culture medium at 1x10<sup>5</sup> cells/mL and infected with HIV at a multiplicity of infection of 0.02. Immediately after virus infection, 100  $\mu$ L of the cell suspension was brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. The test compounds were dissolved in DMSO at 50 mM or higher. After 4-days incubation at 37 °C, the number of viable cells was determined using the MTT method. Compounds were tested in parallel for cytotoxic effects in uninfected MT-4 cells. The drugs currently being used in clinical treatment of HIV-1 infection, DDN/DDC and DMP266 were used as control.

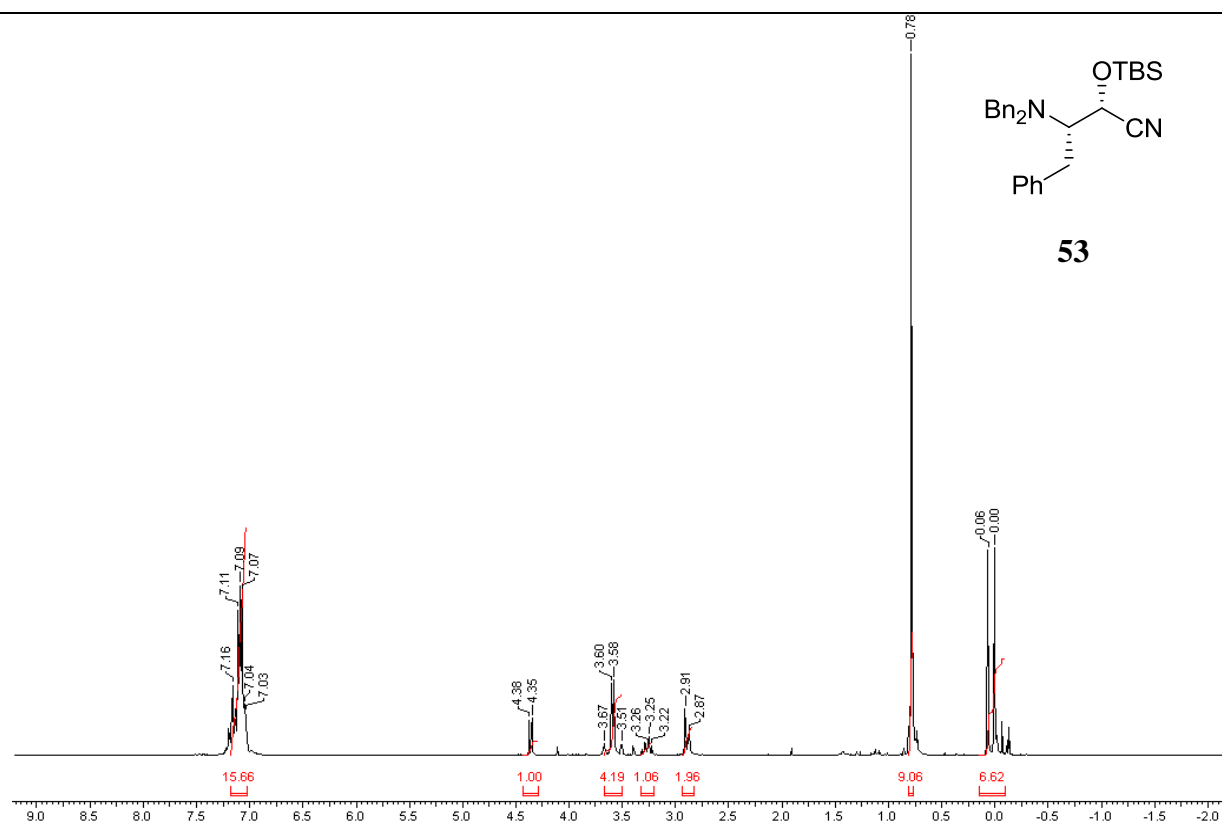
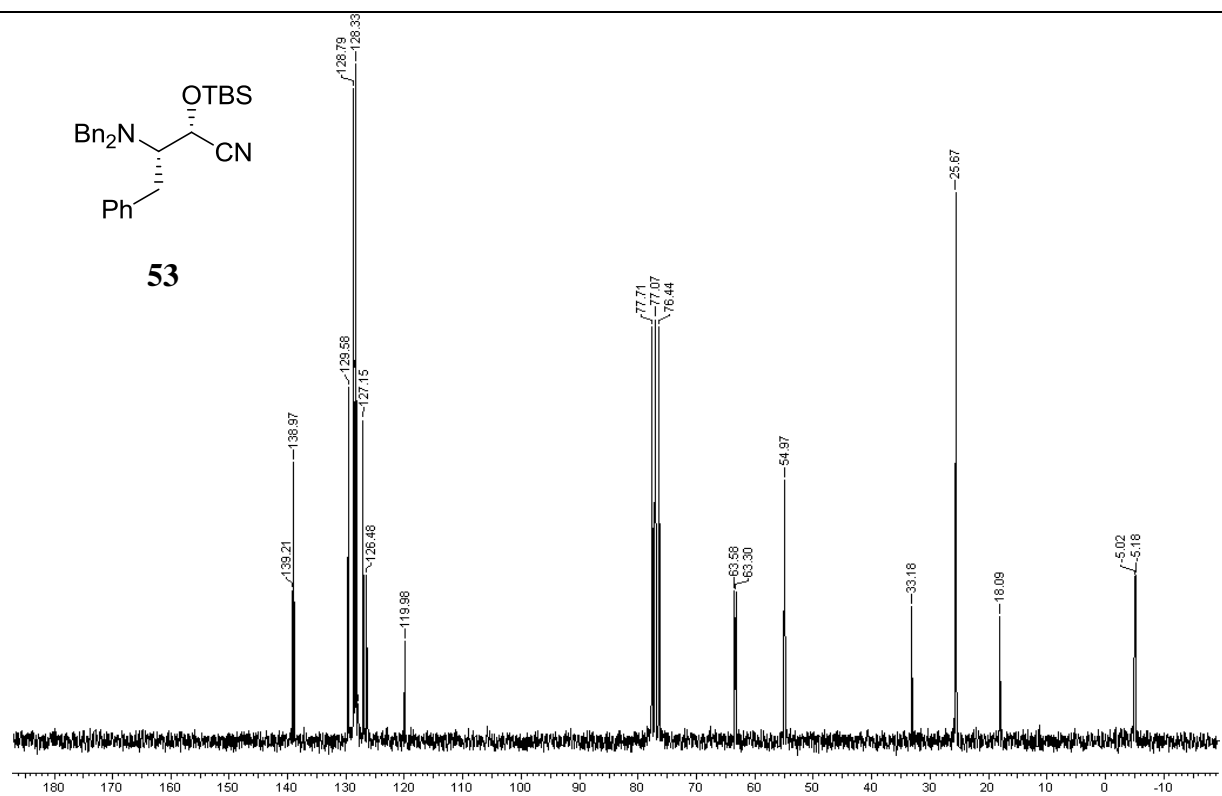
## Spectra

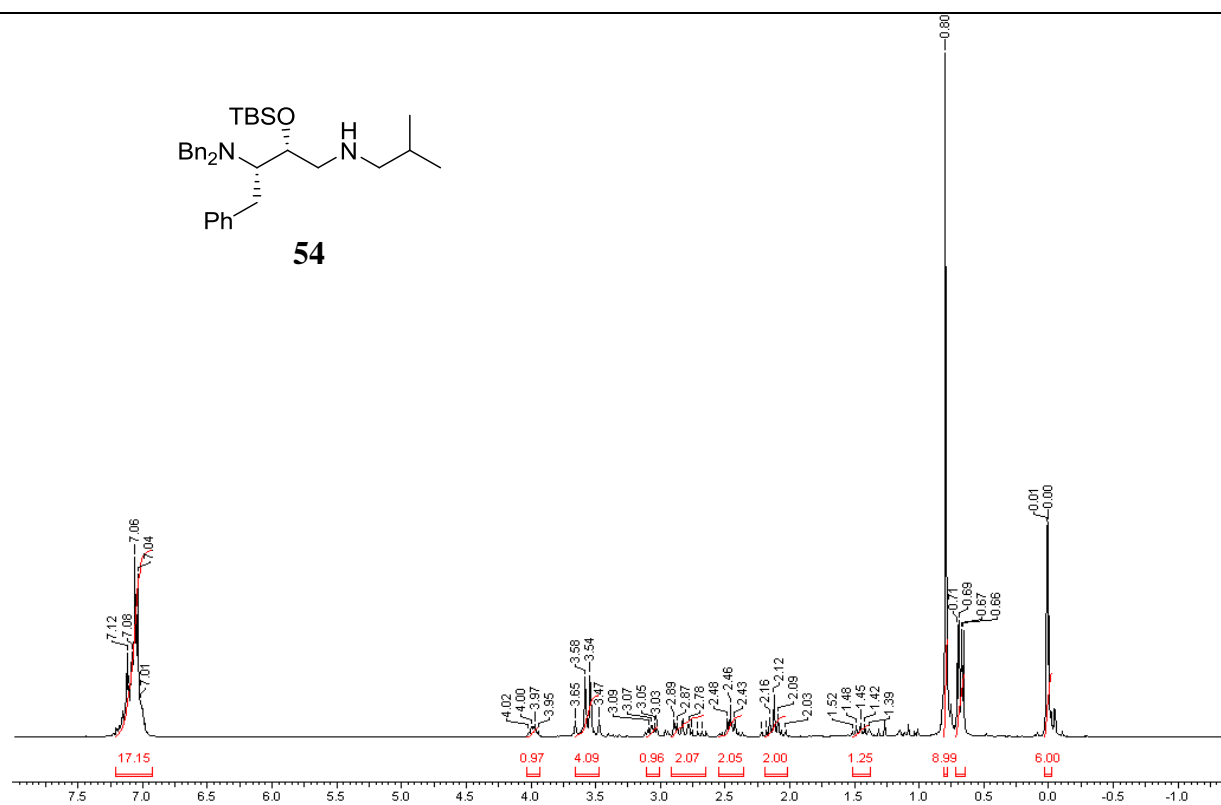
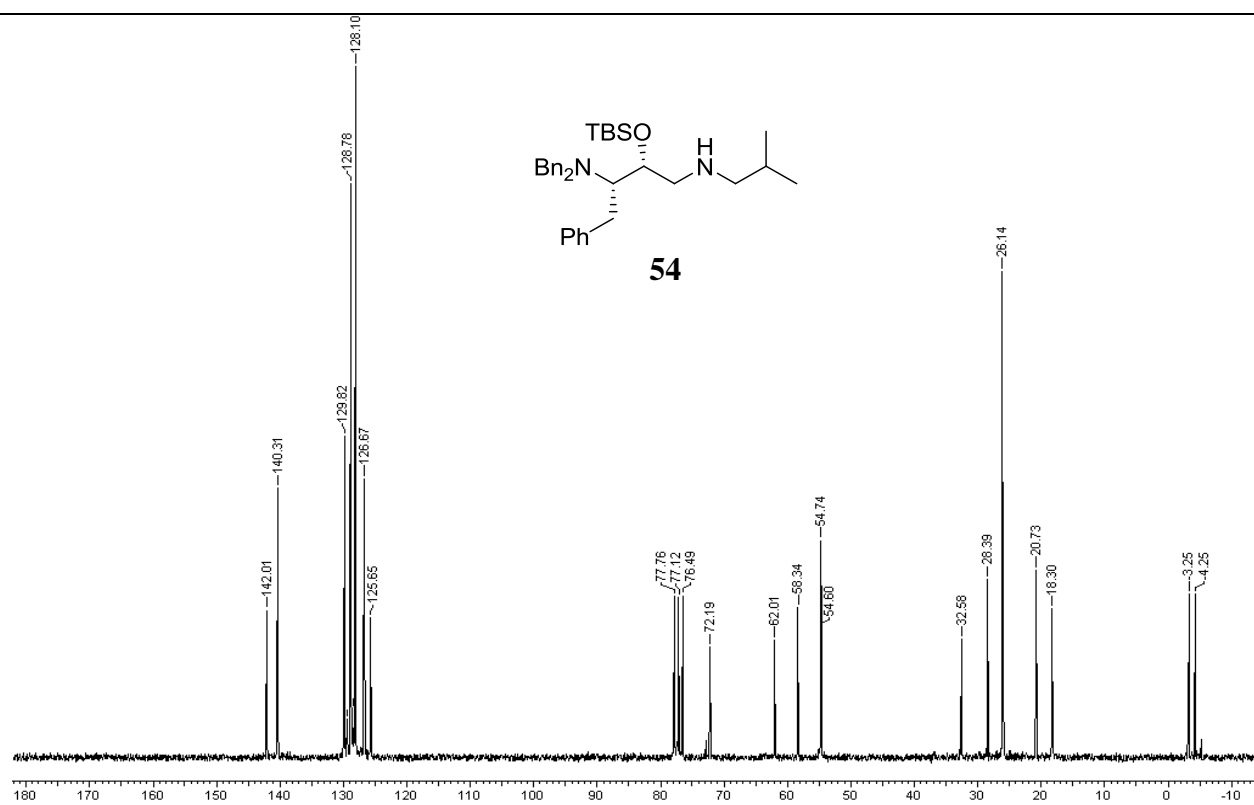
<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **44**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **44**

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **47**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **47**

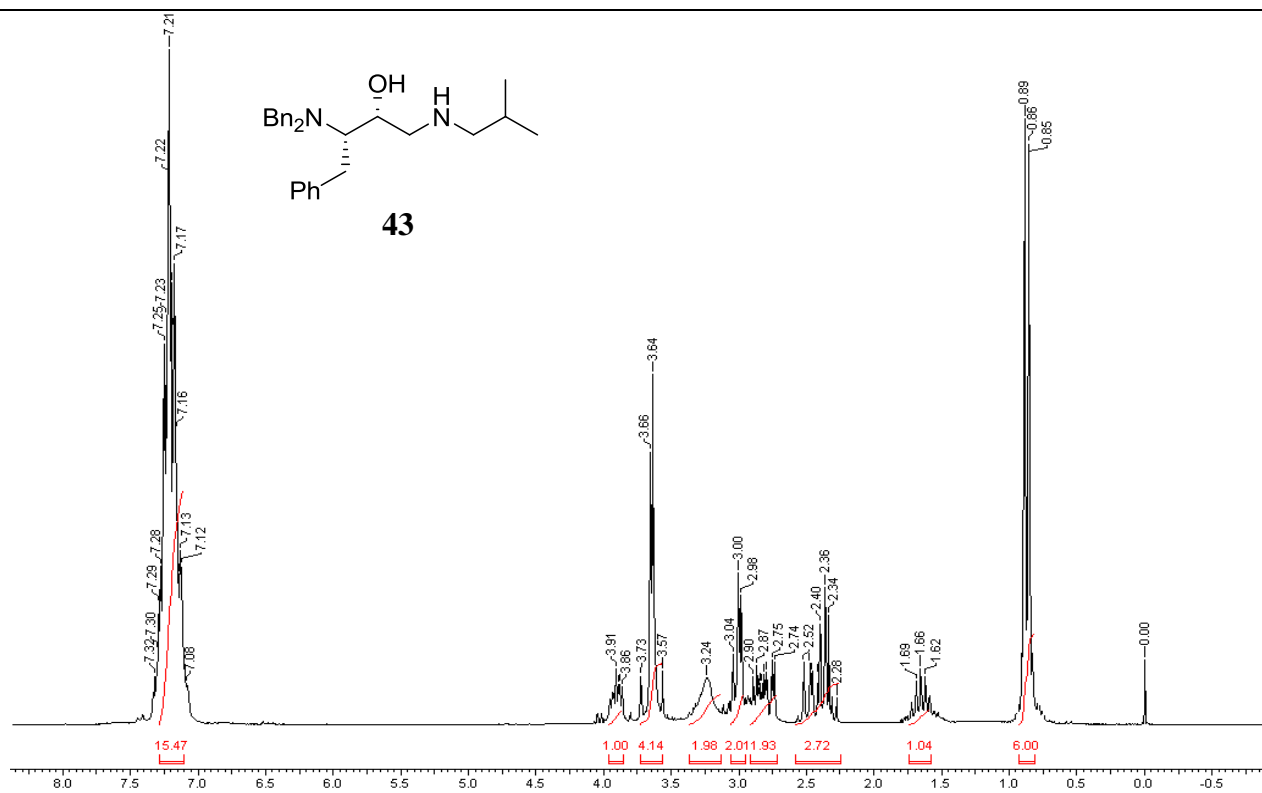
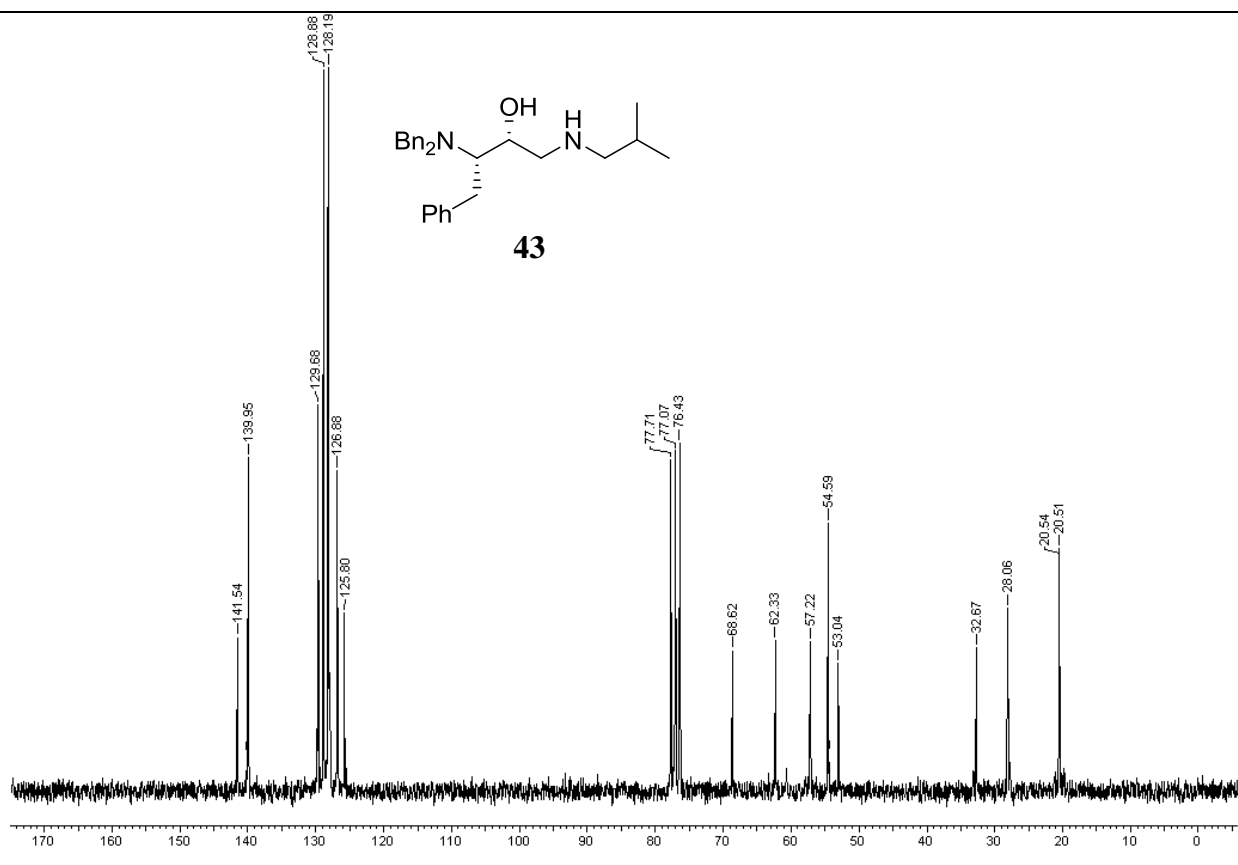
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **51a** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **51a**

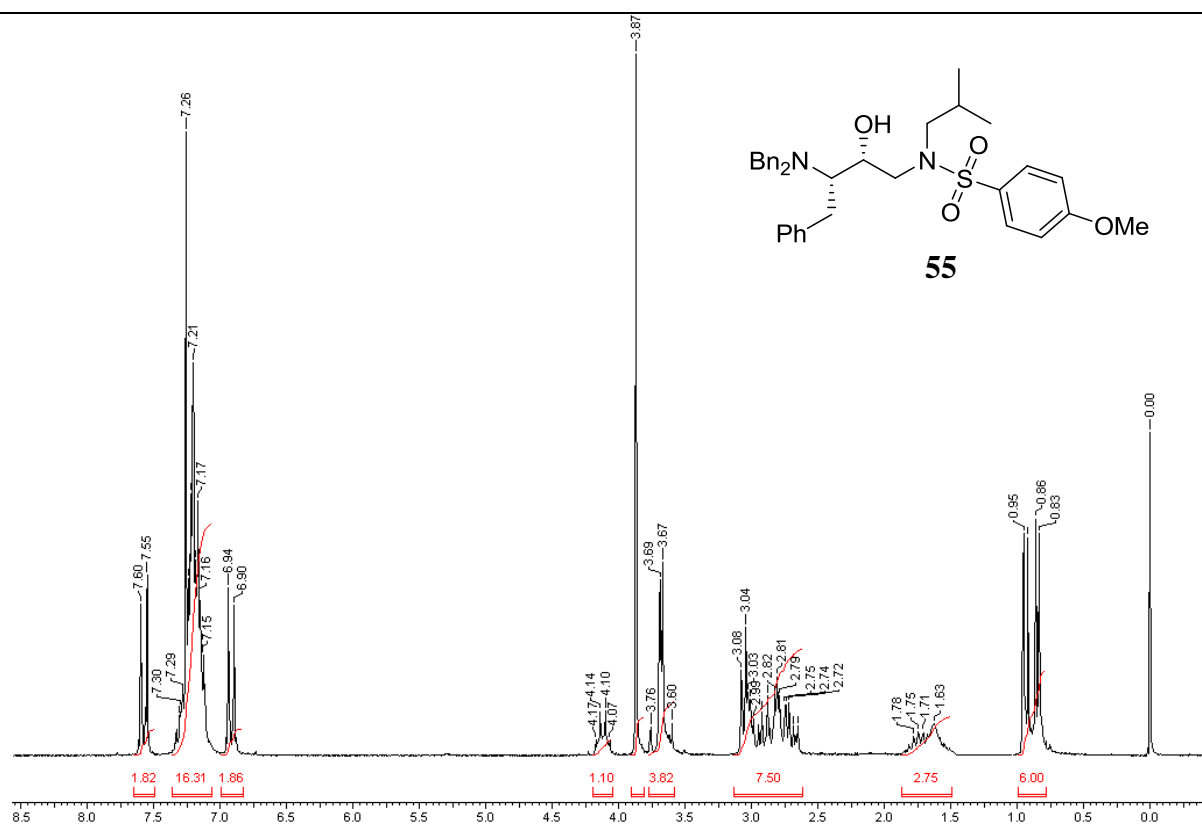
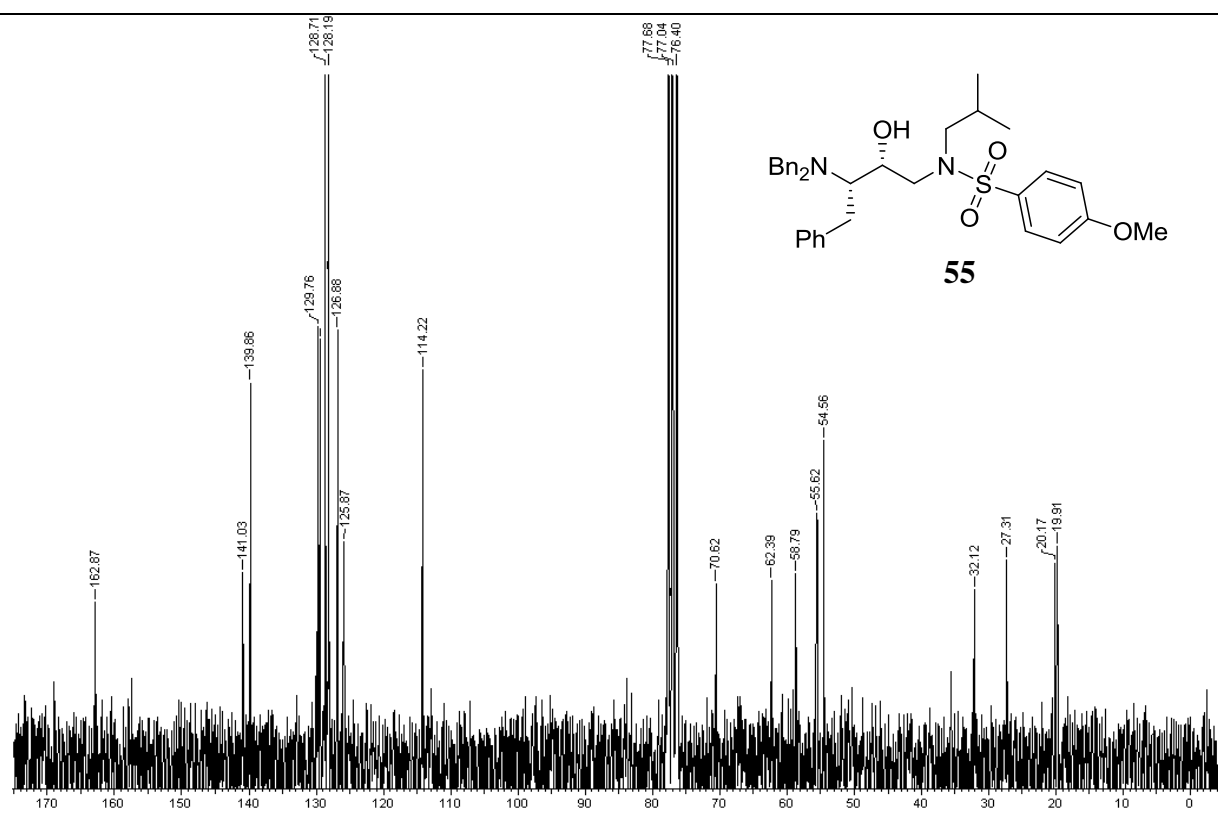
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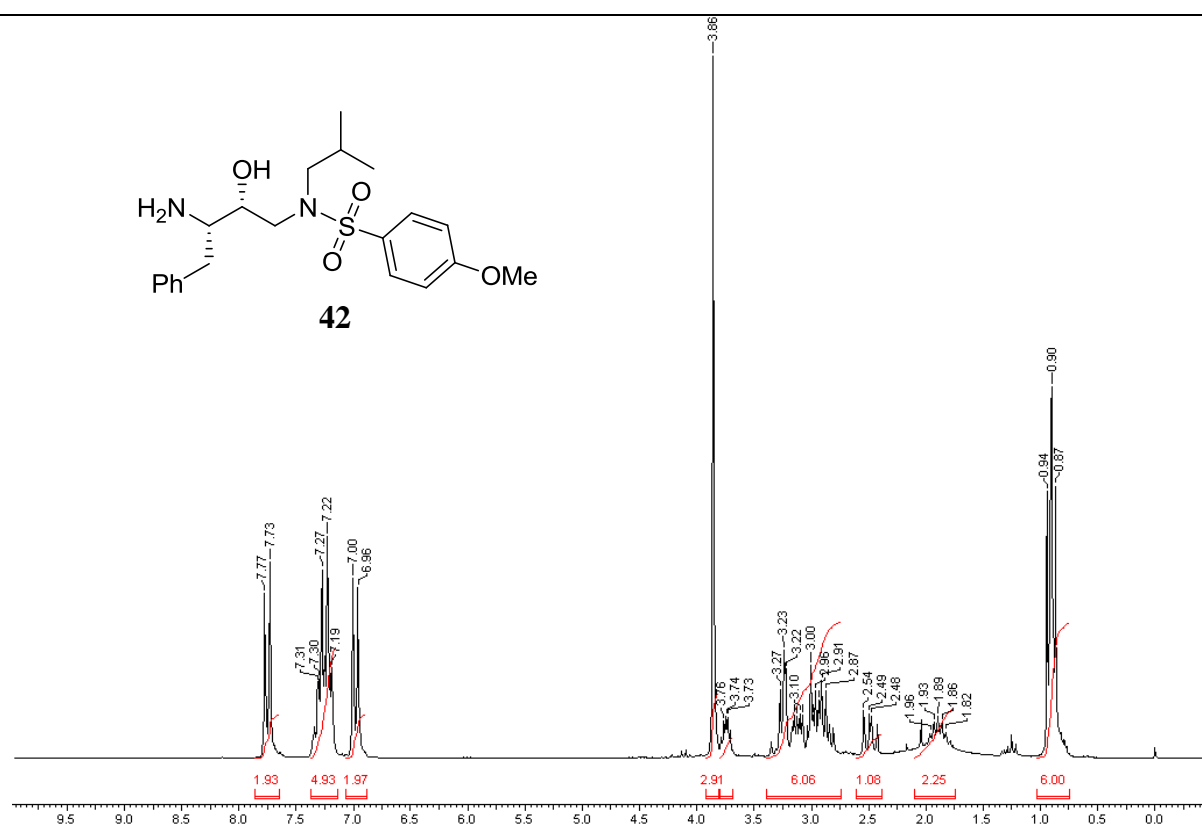
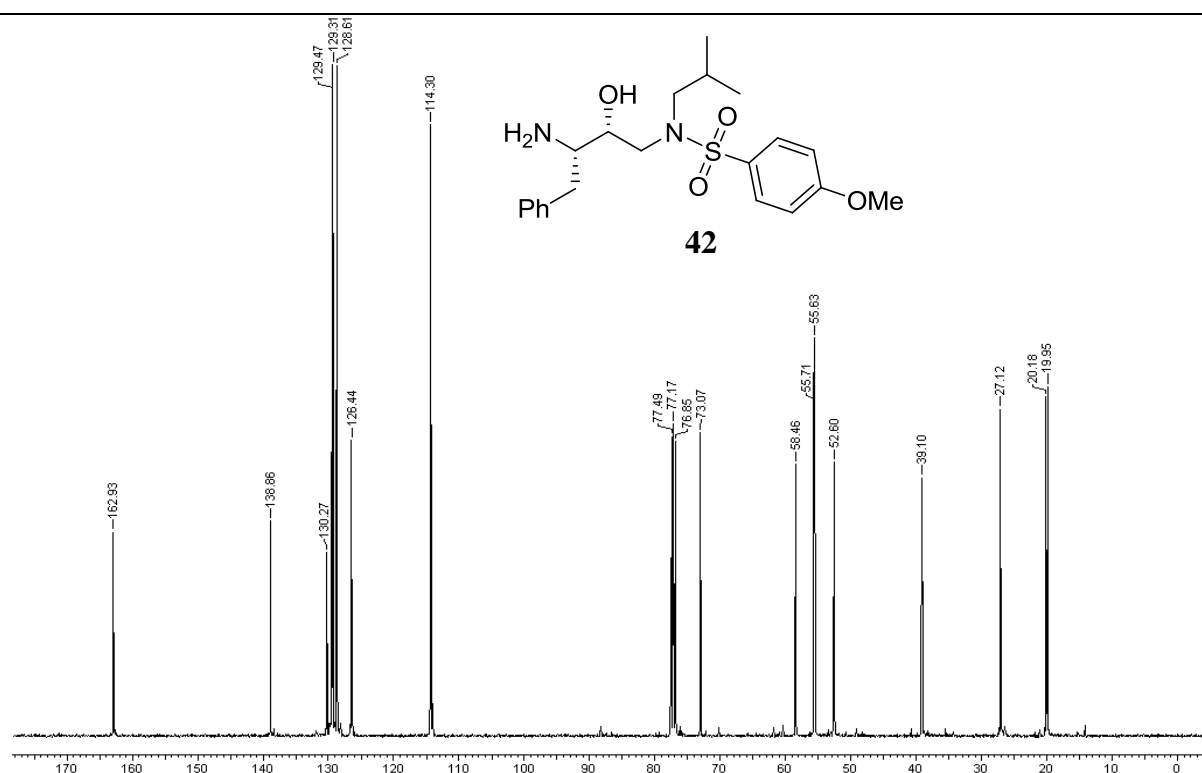
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **53** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **53**

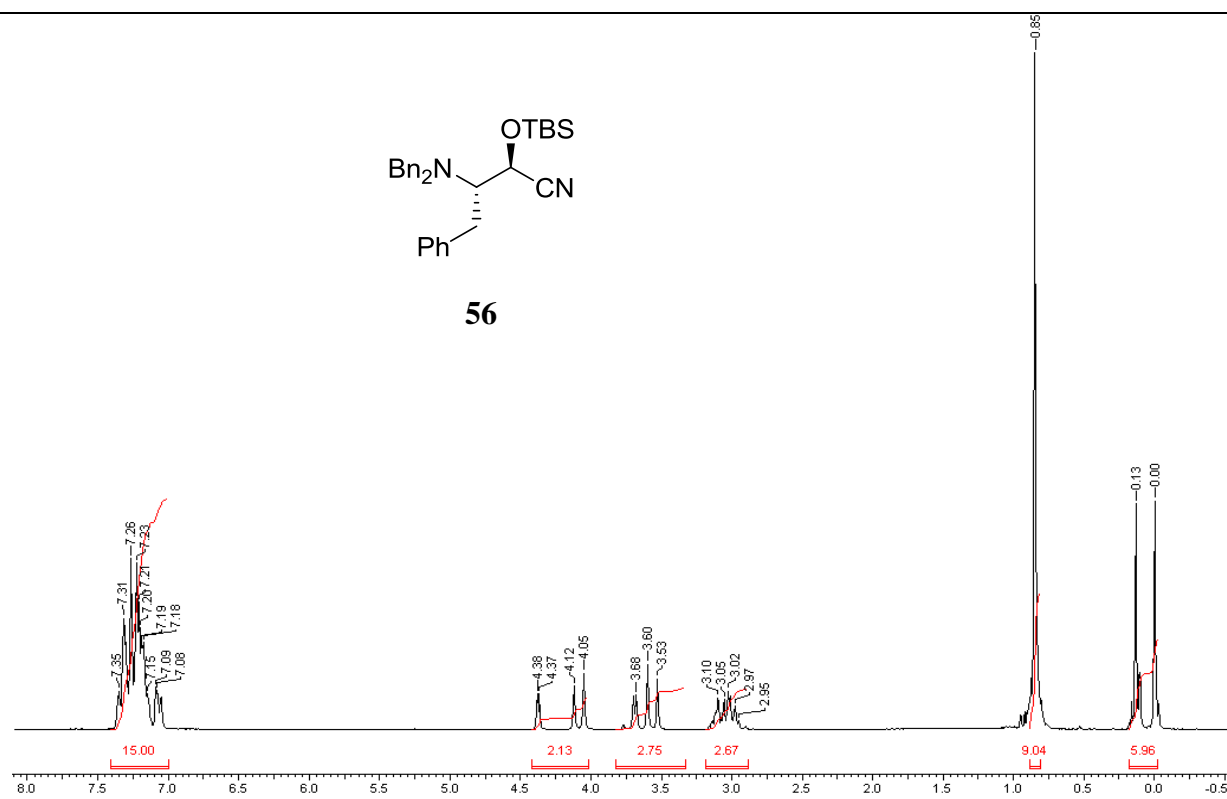
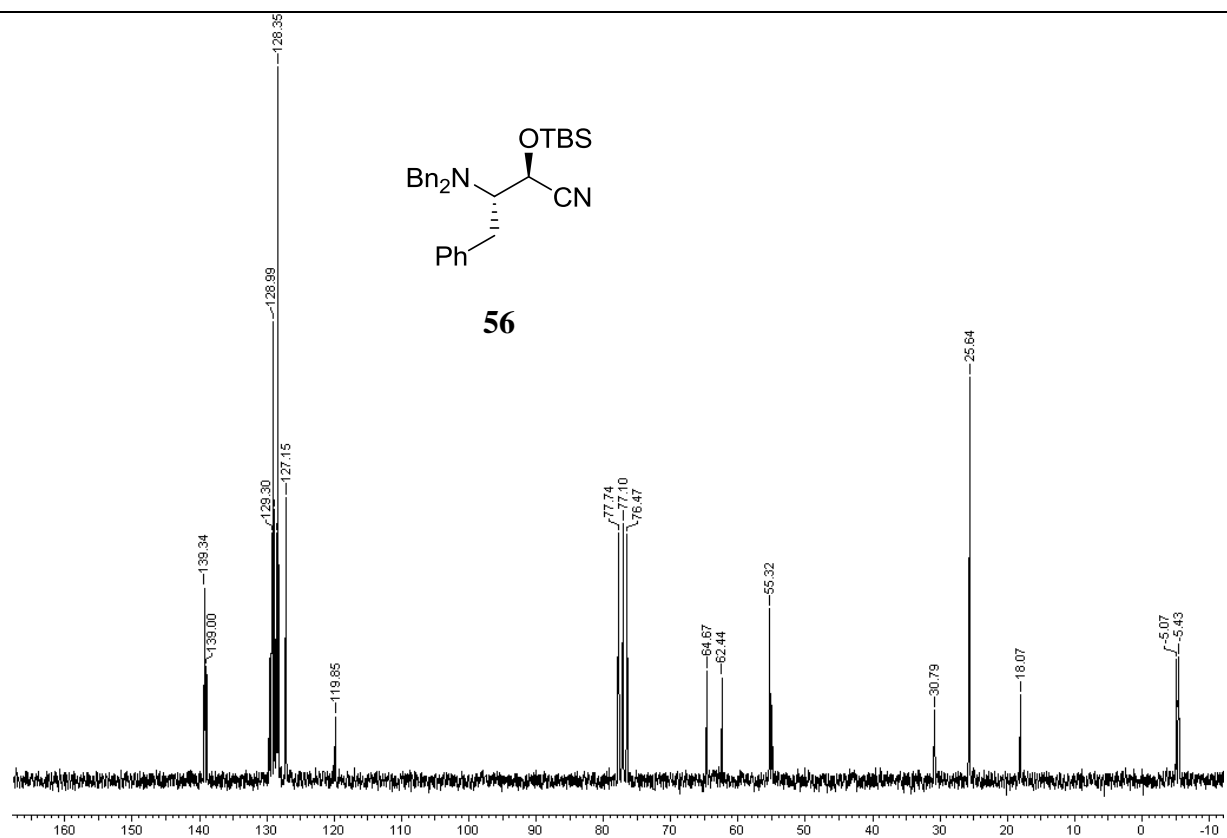
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **54** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **54**

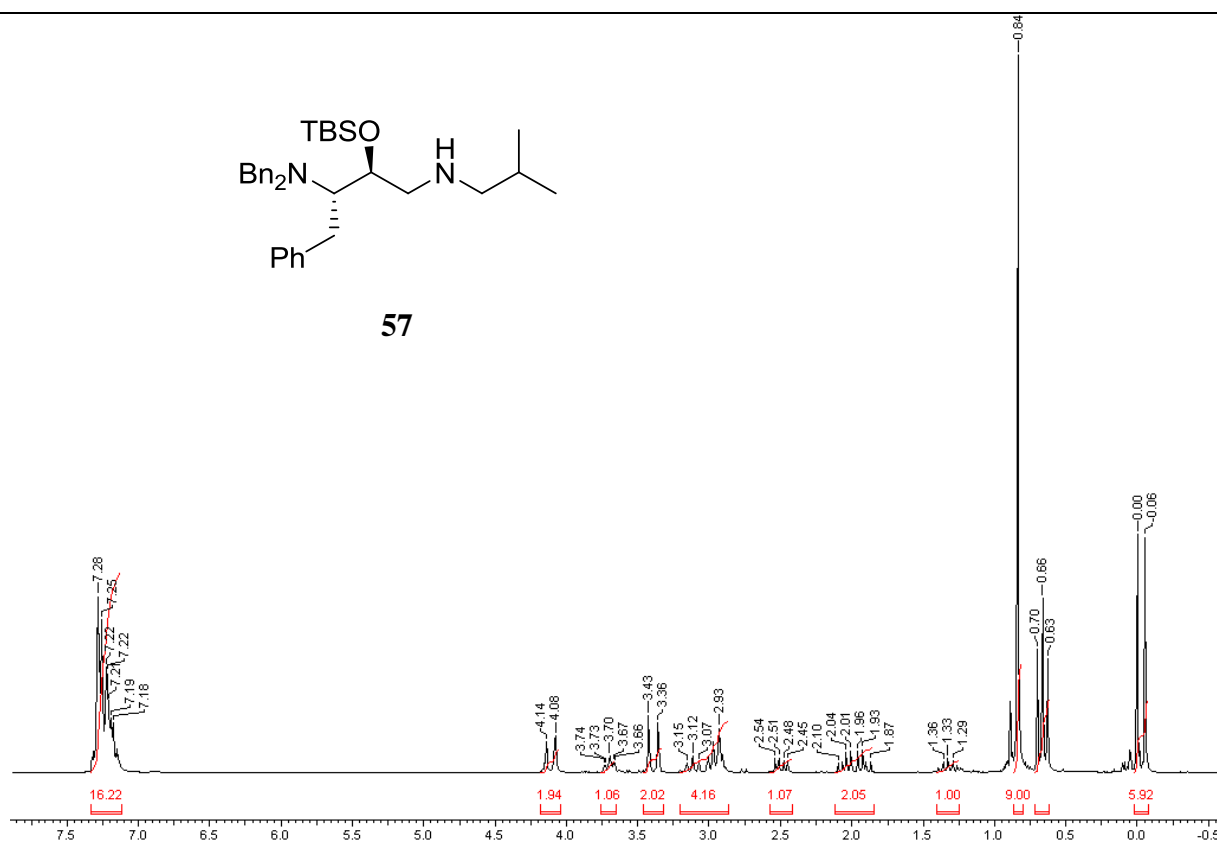
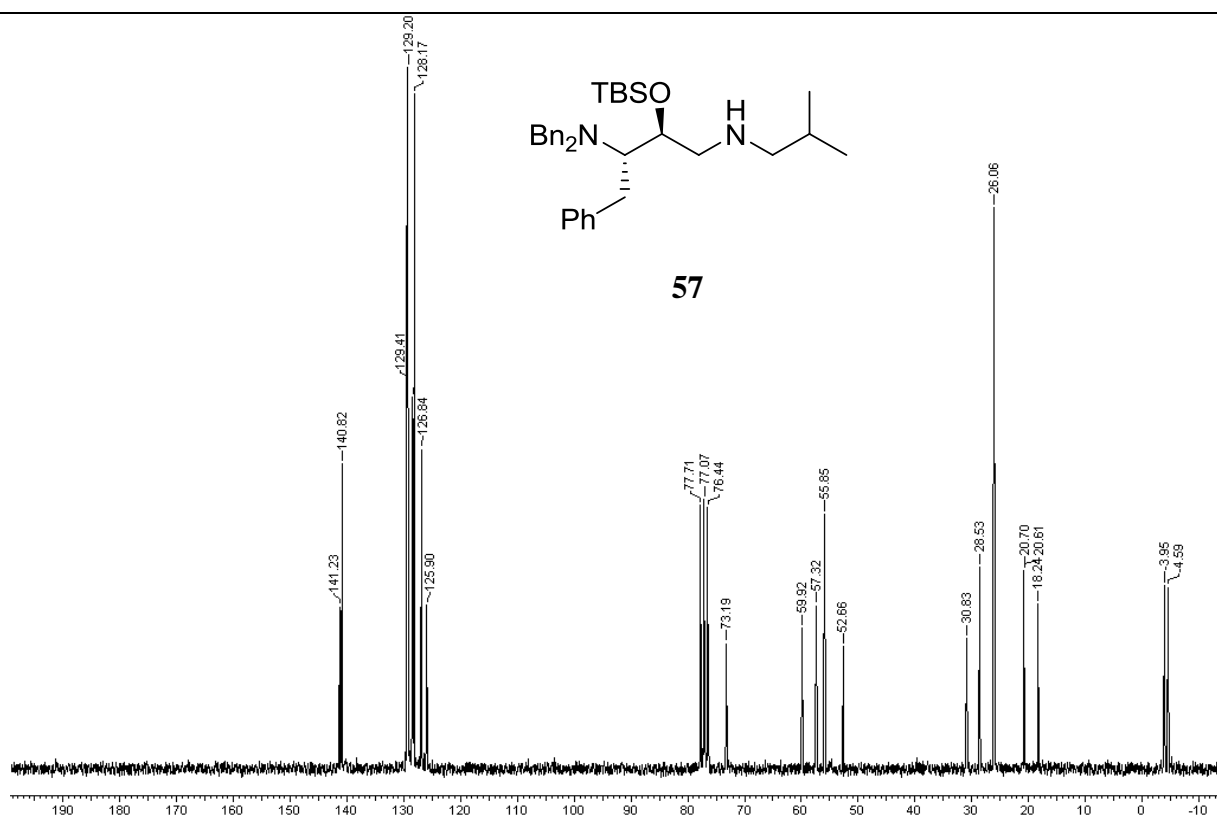


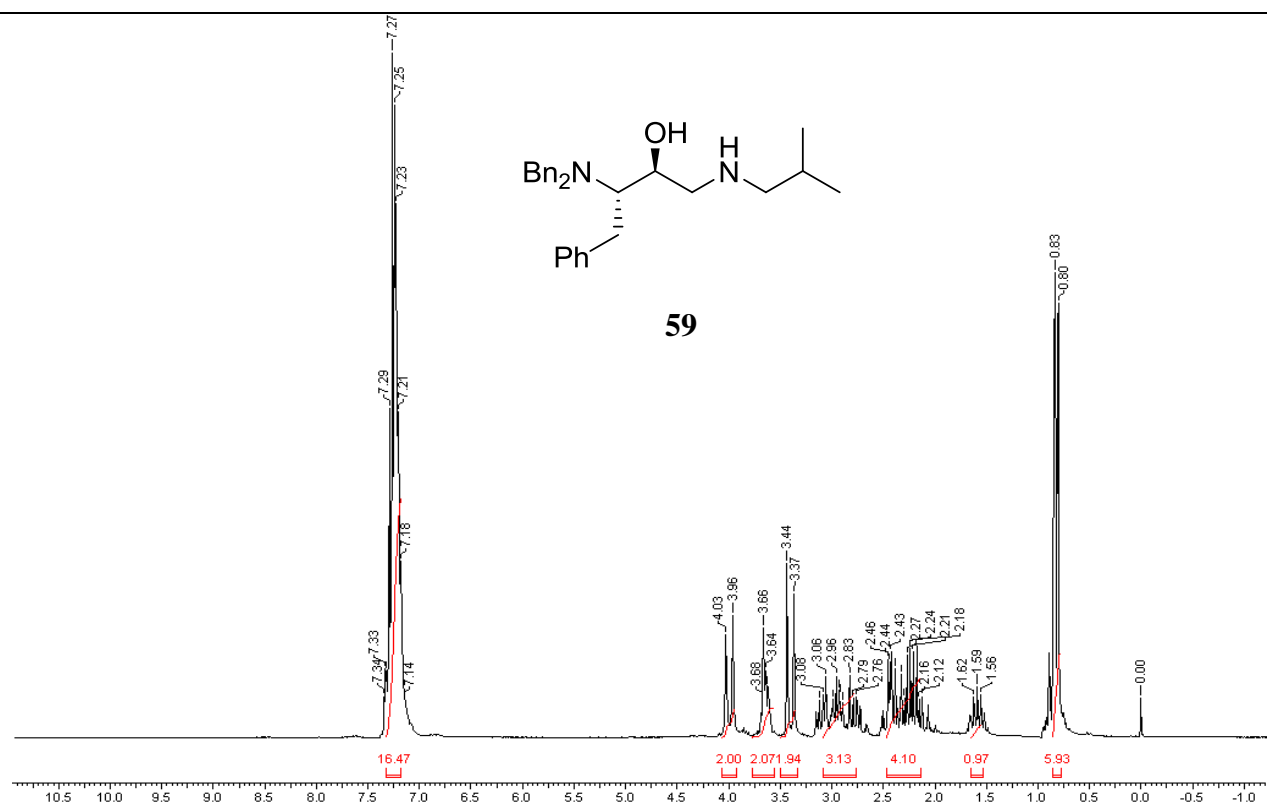
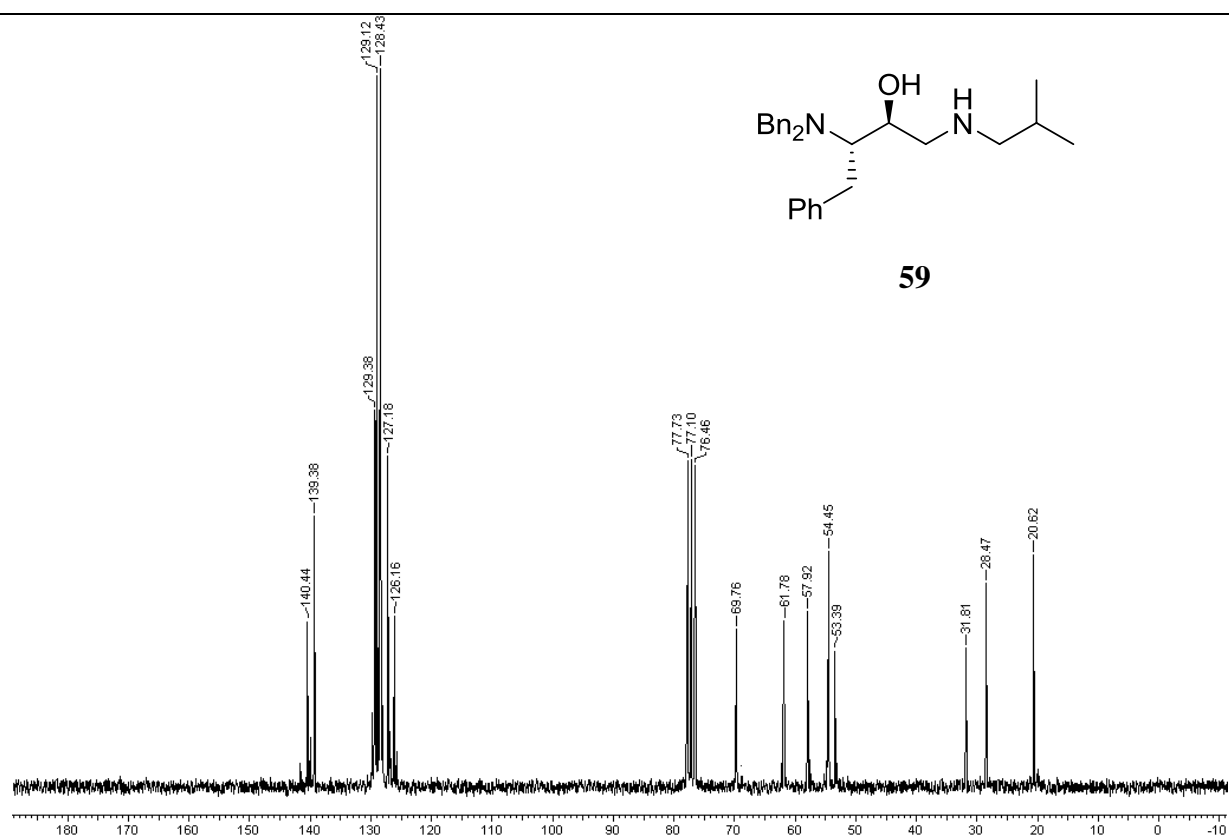
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **43** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **43**

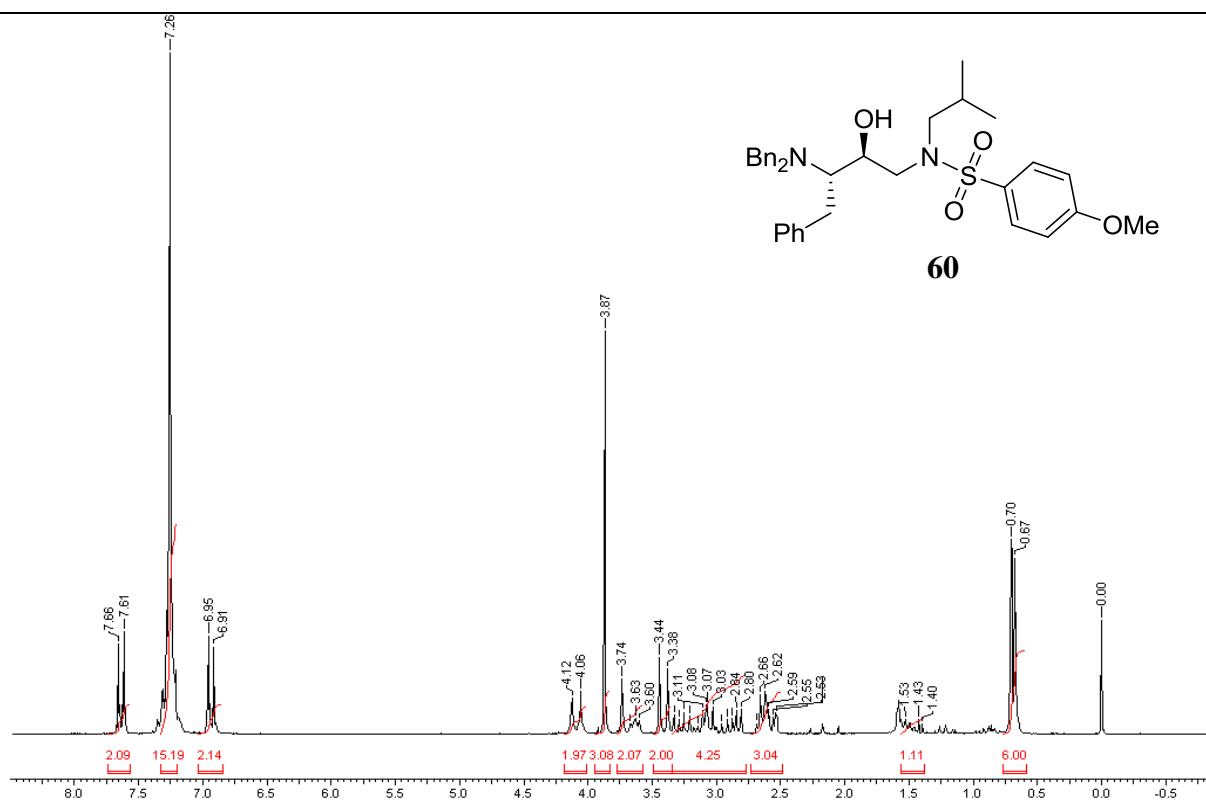
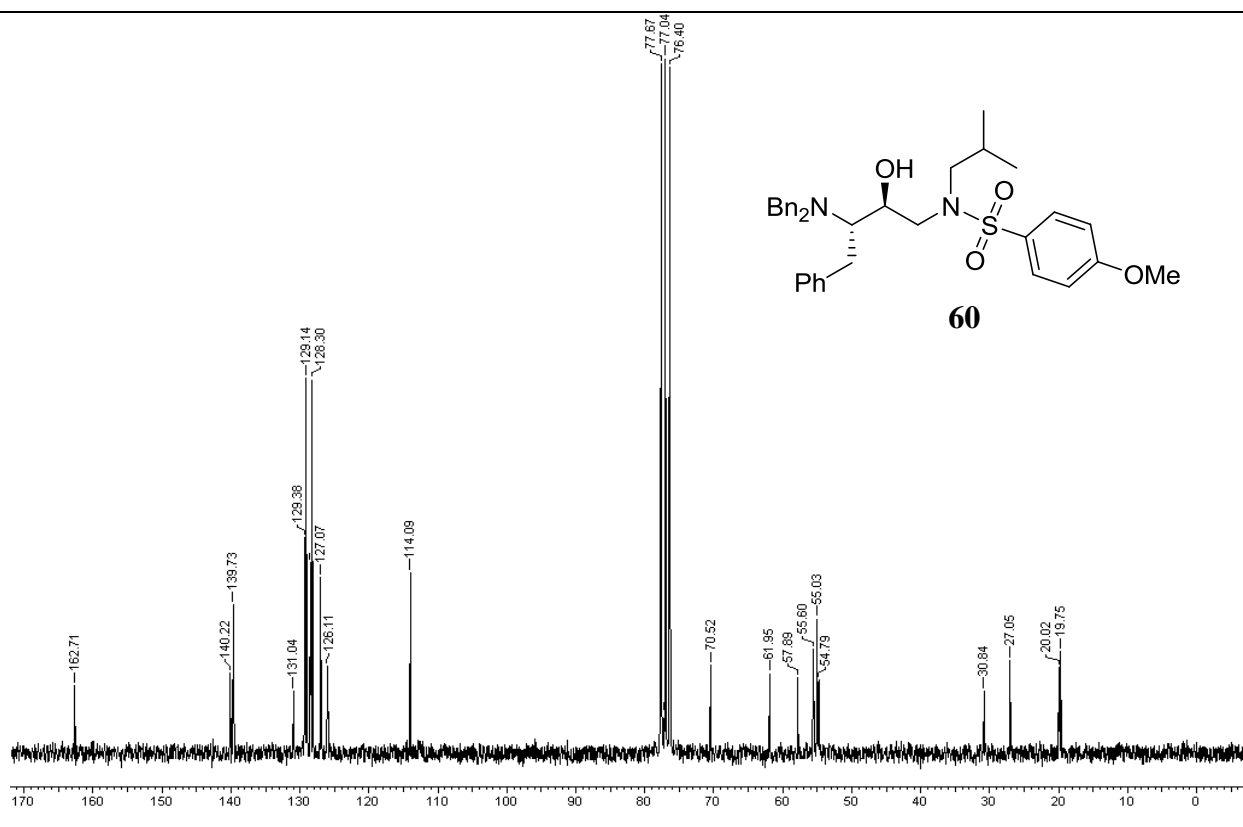
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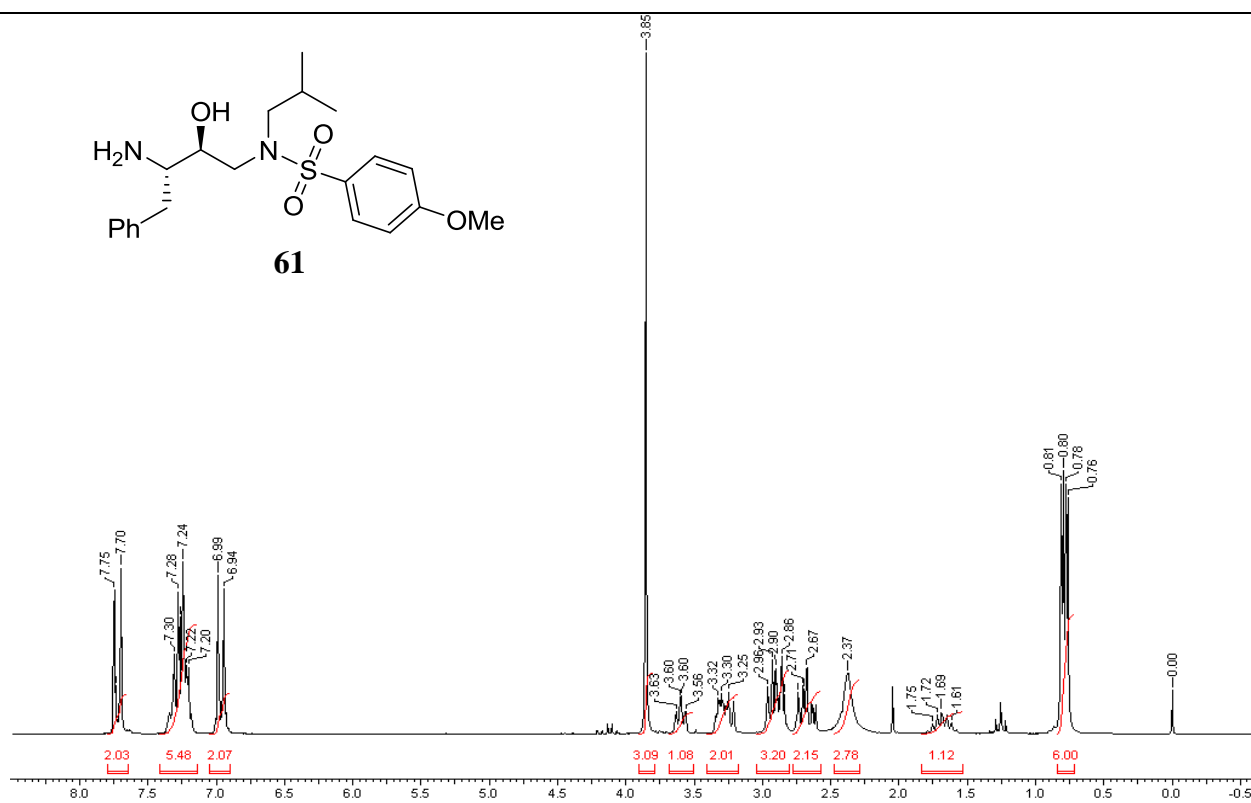
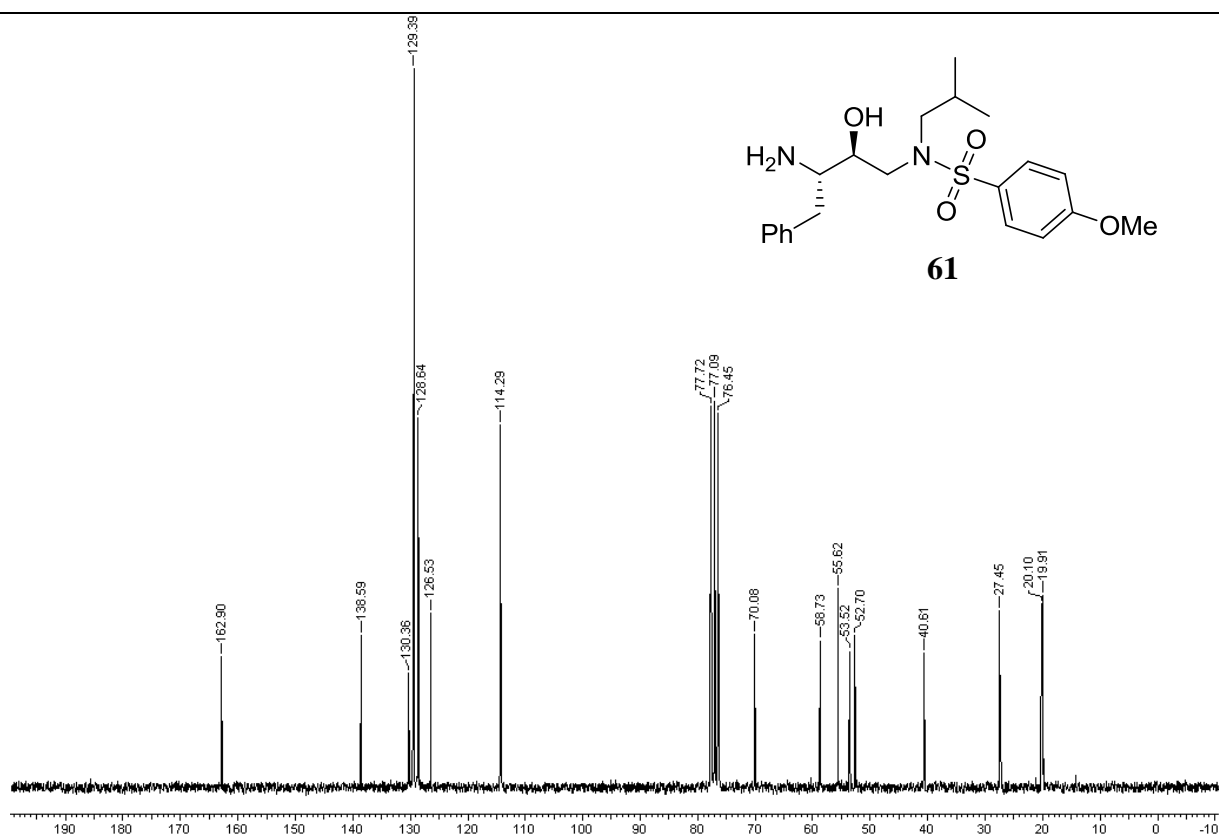
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **42** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **42**

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **56** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **56**

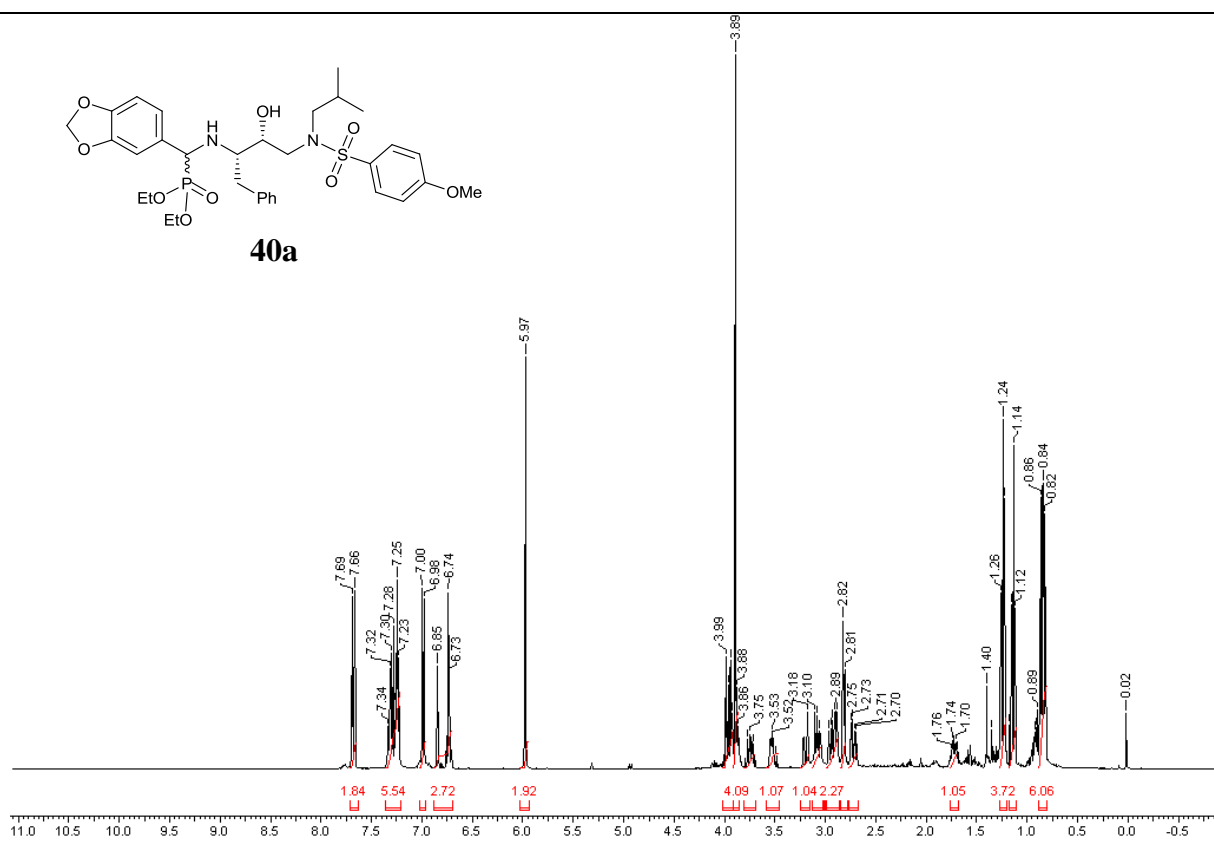
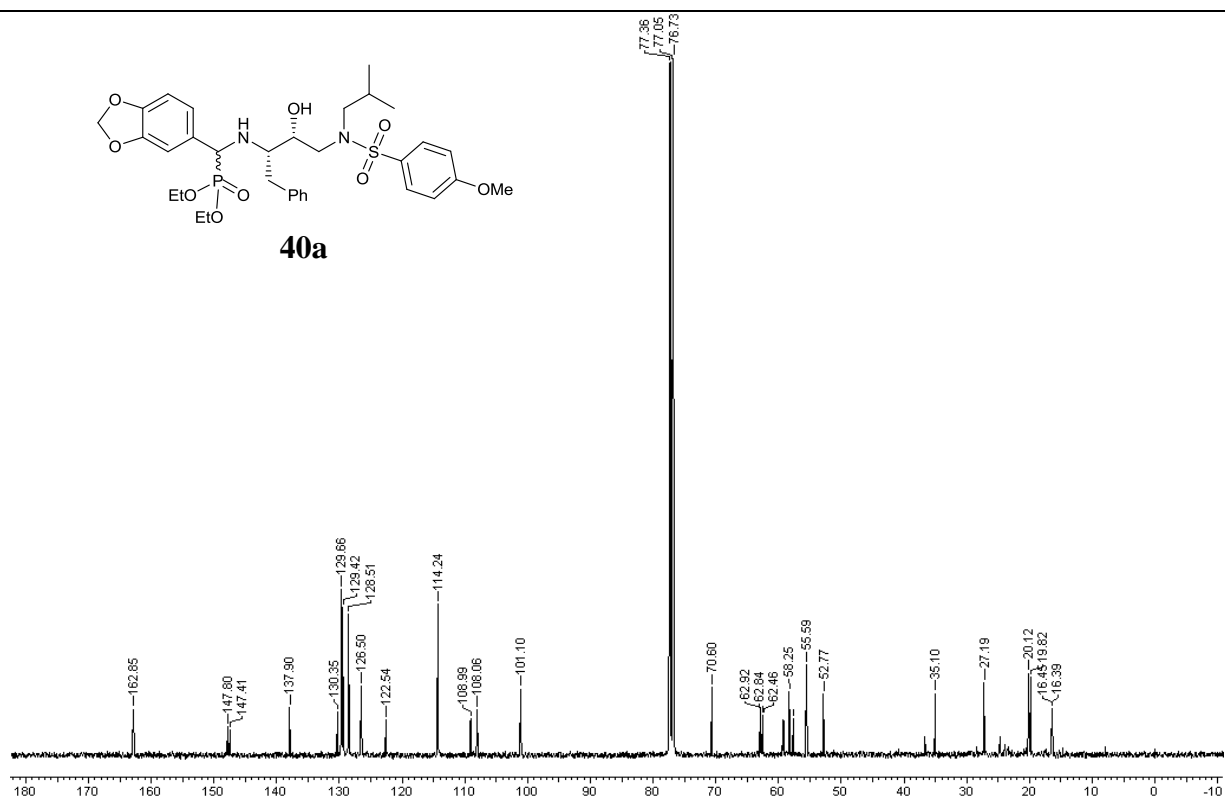
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **57** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **57**

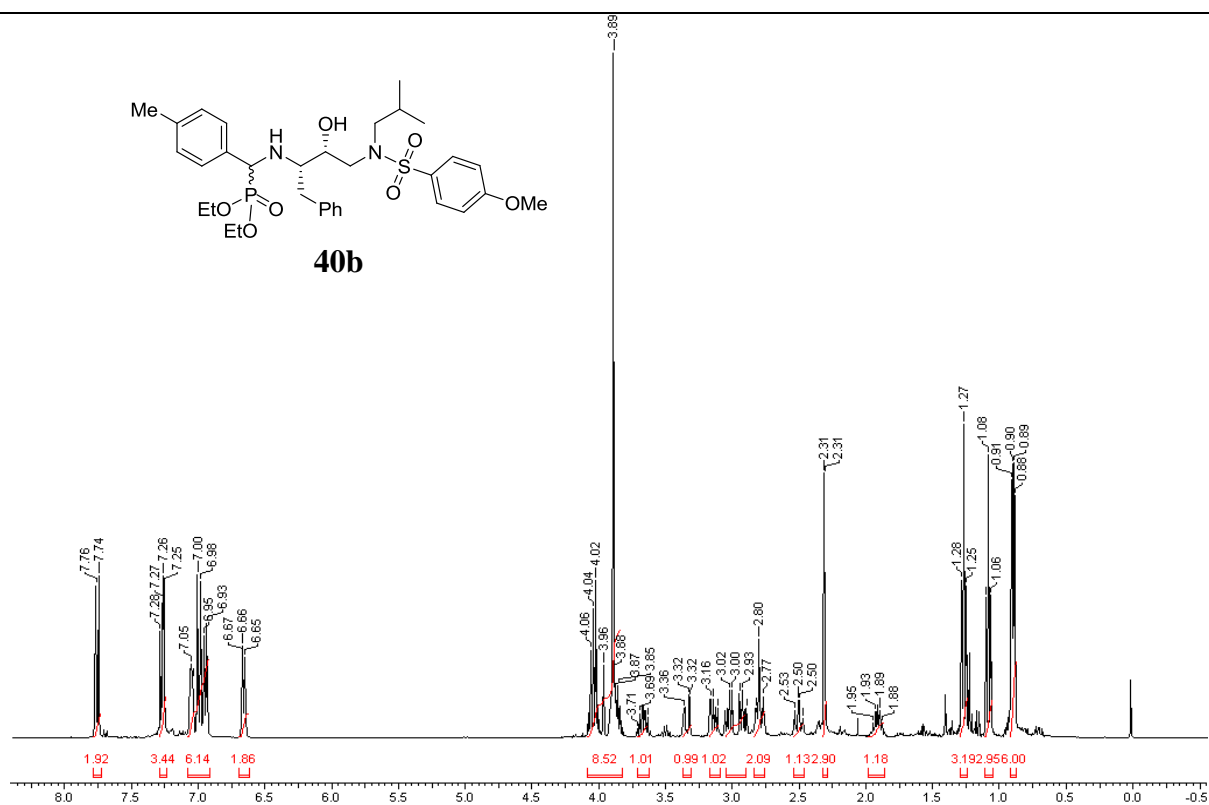
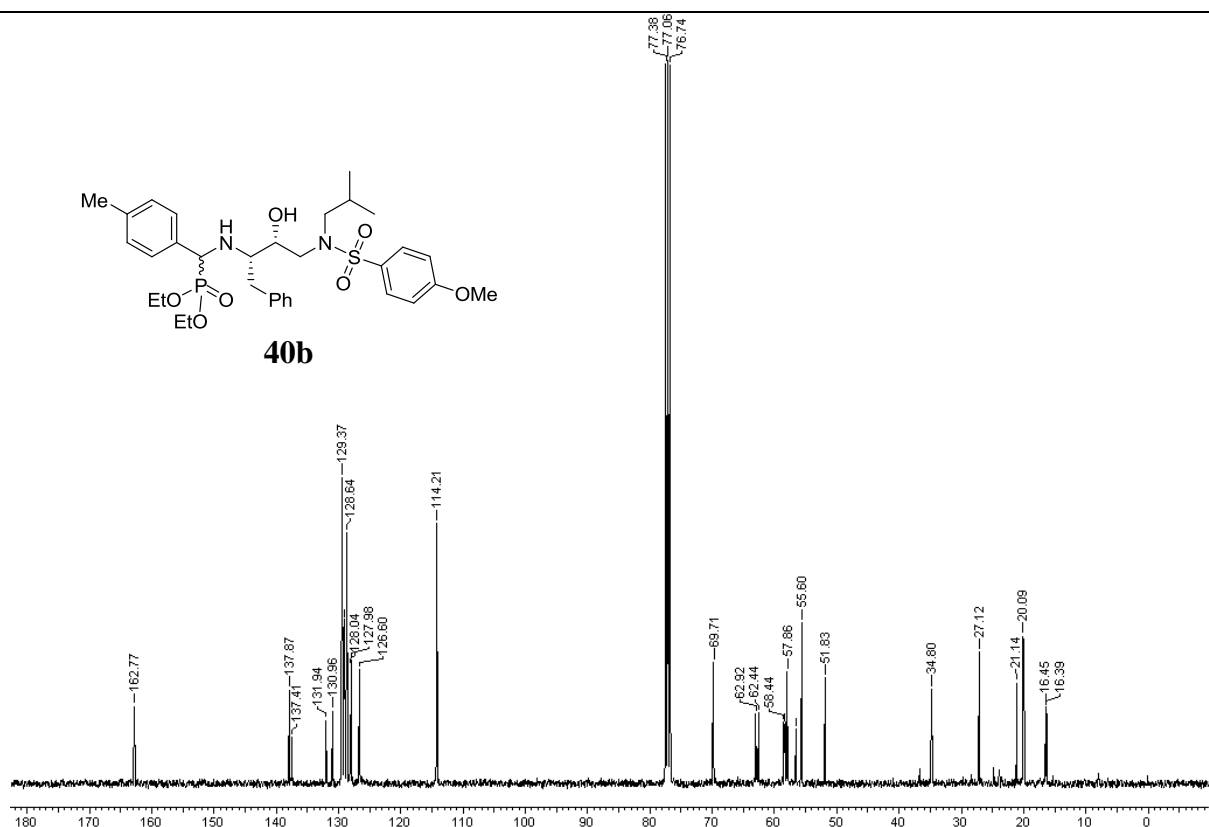
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **59** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **59**

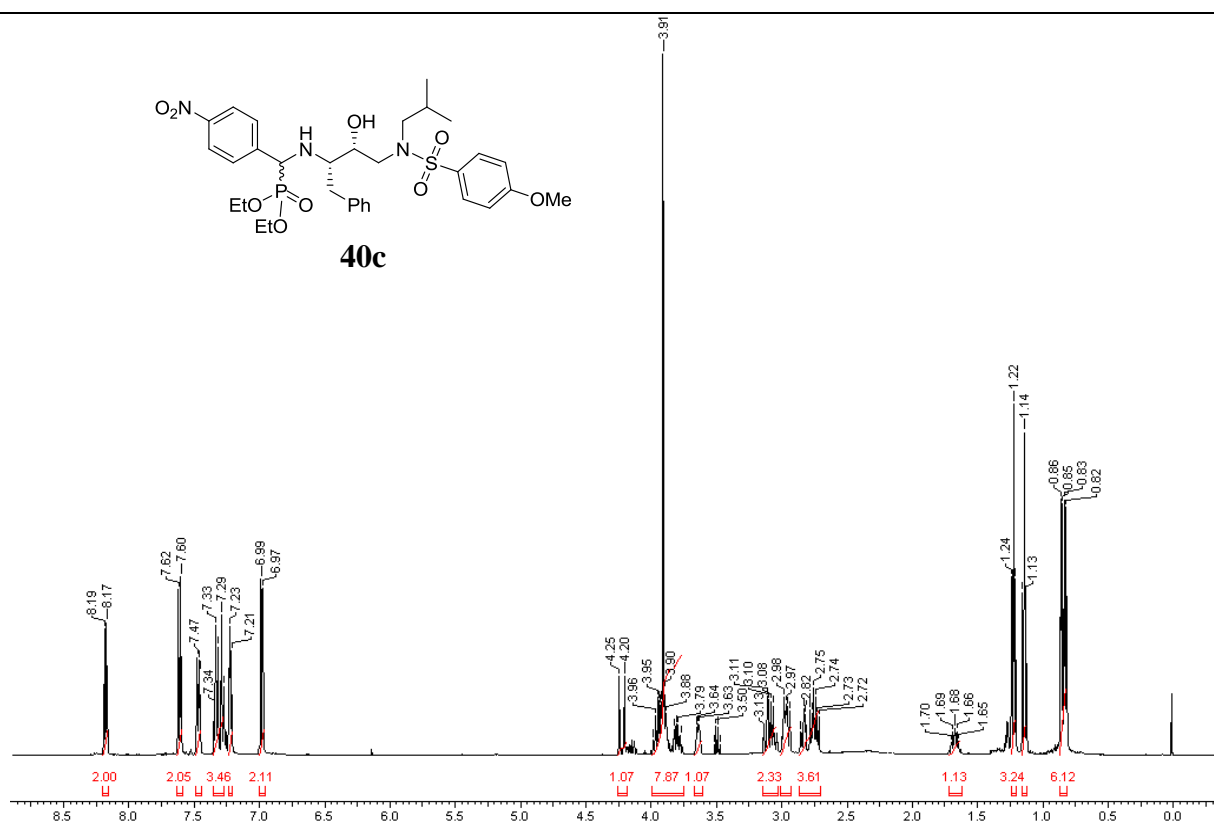
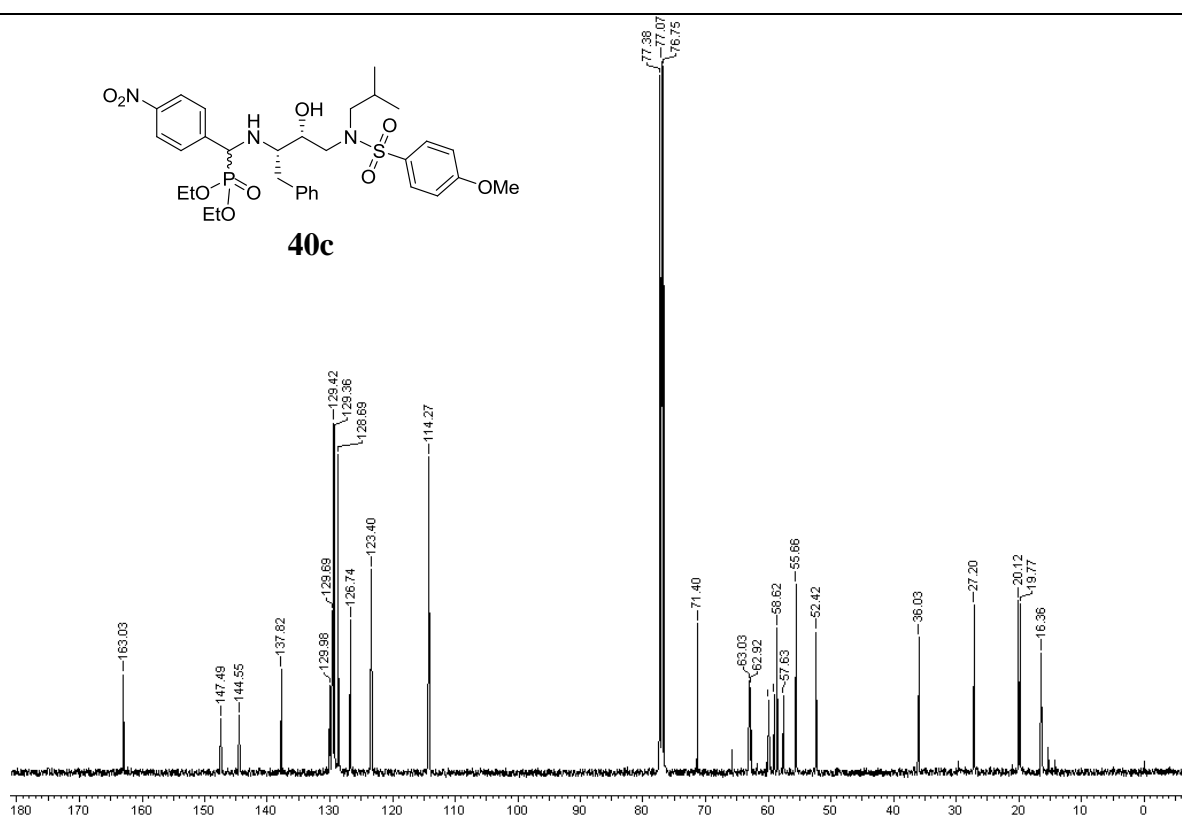
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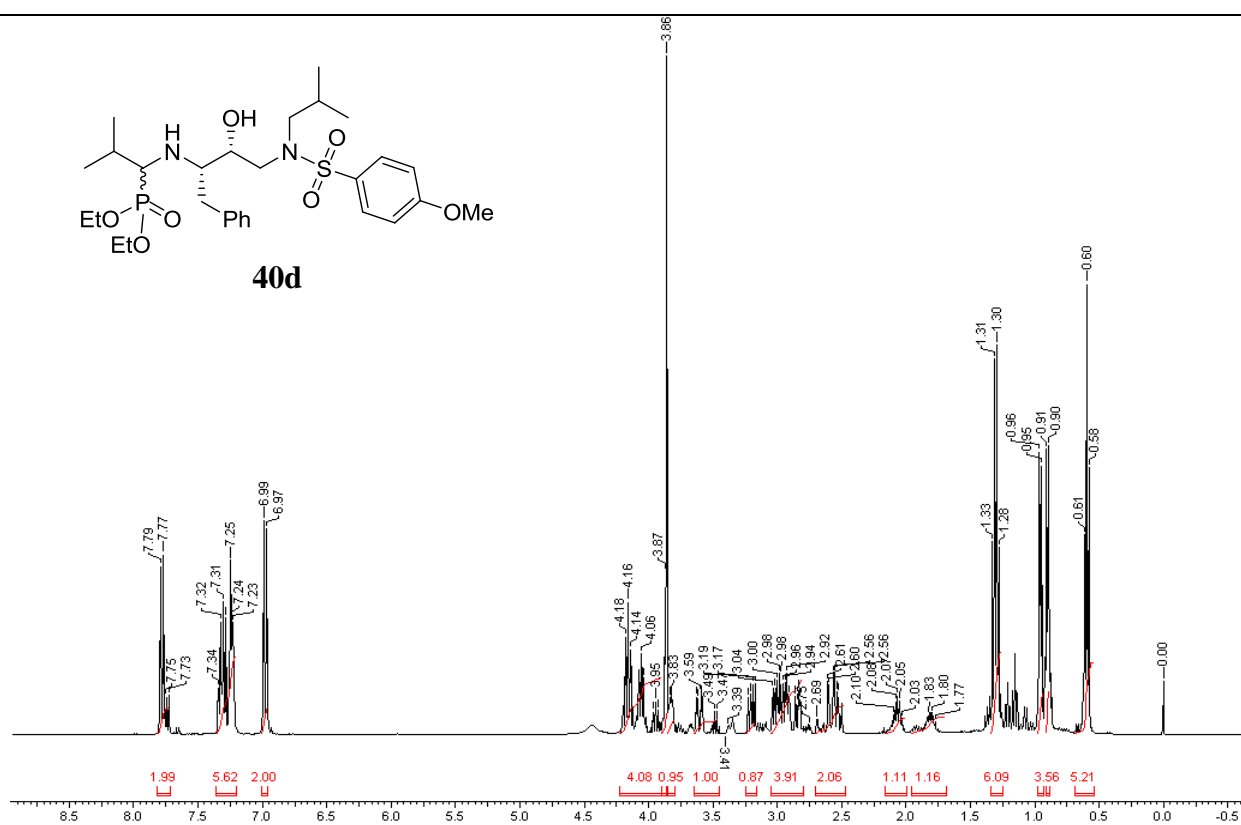
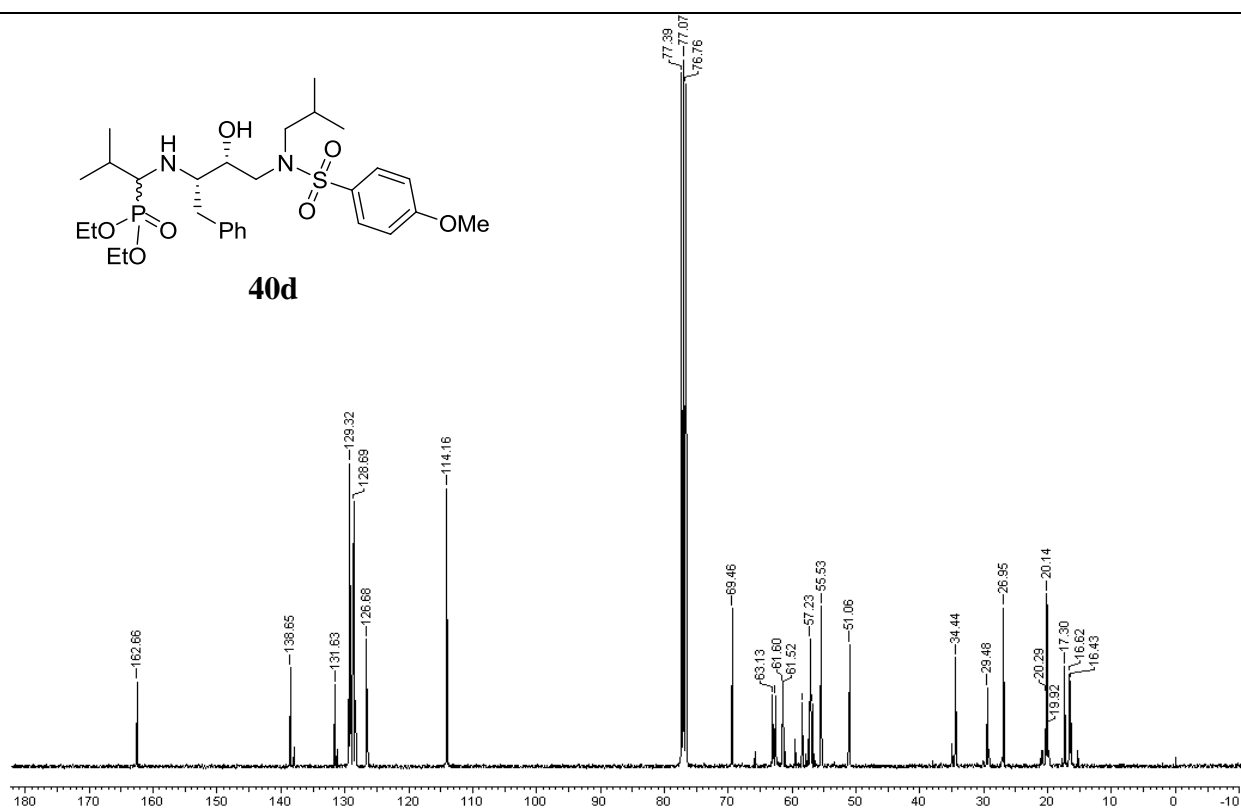
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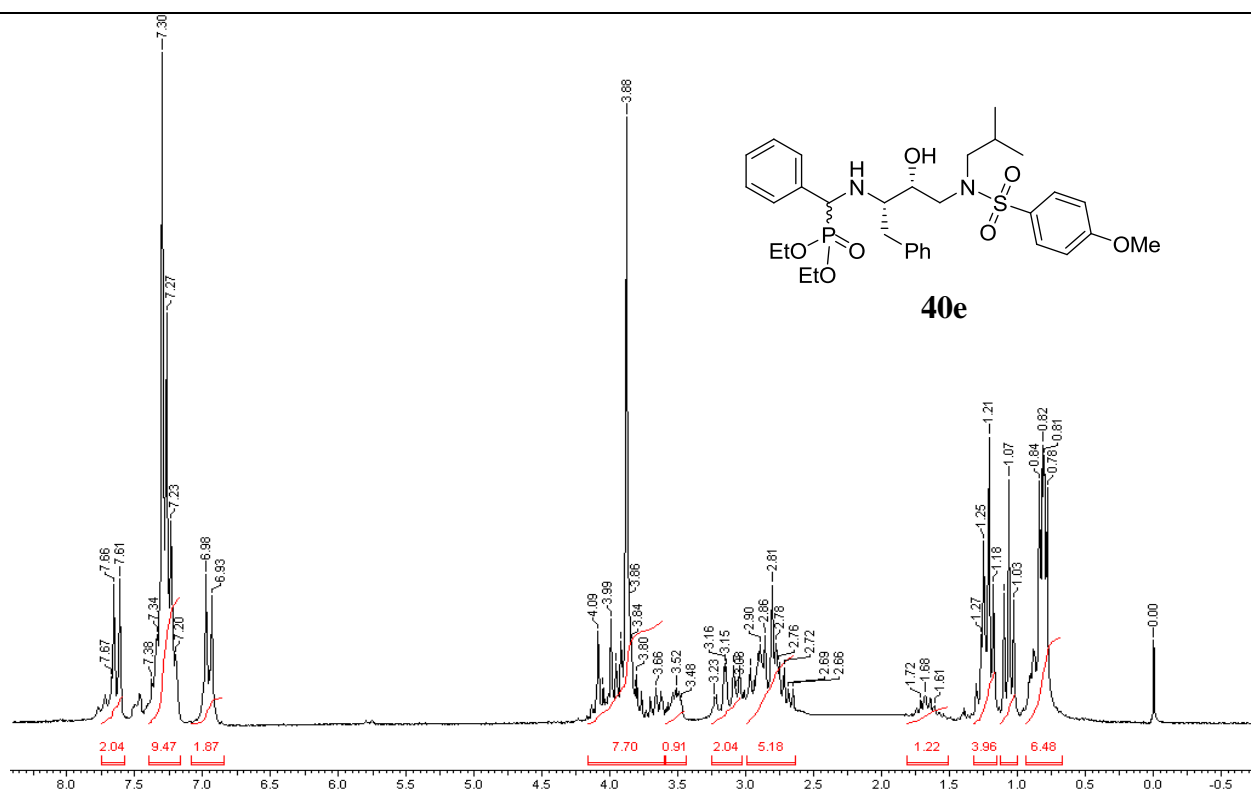
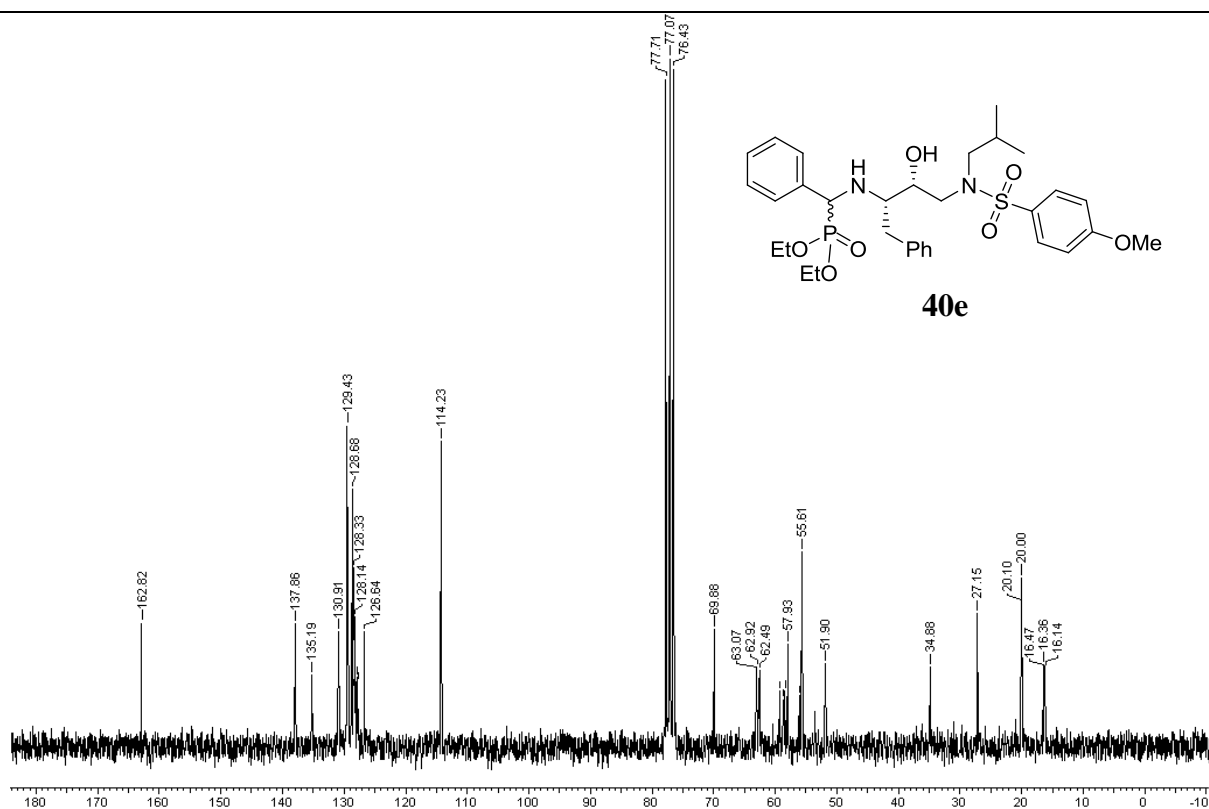


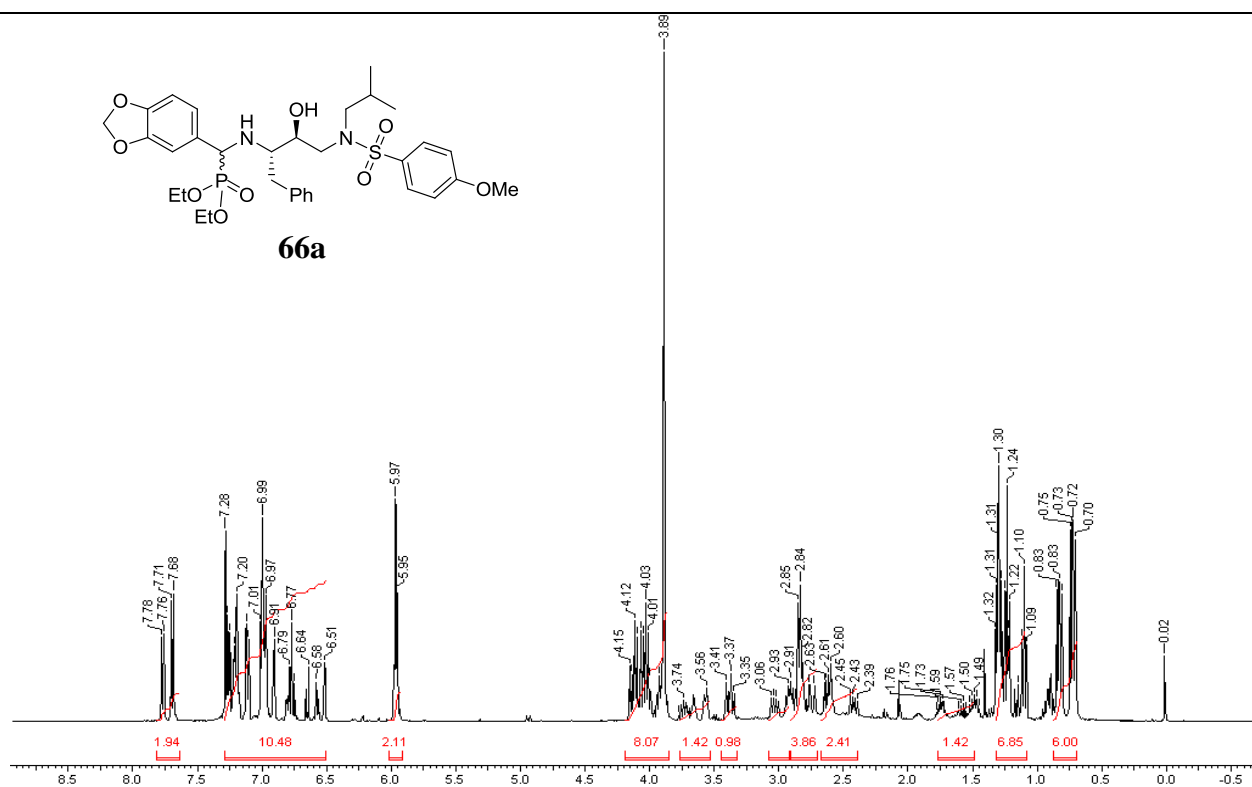
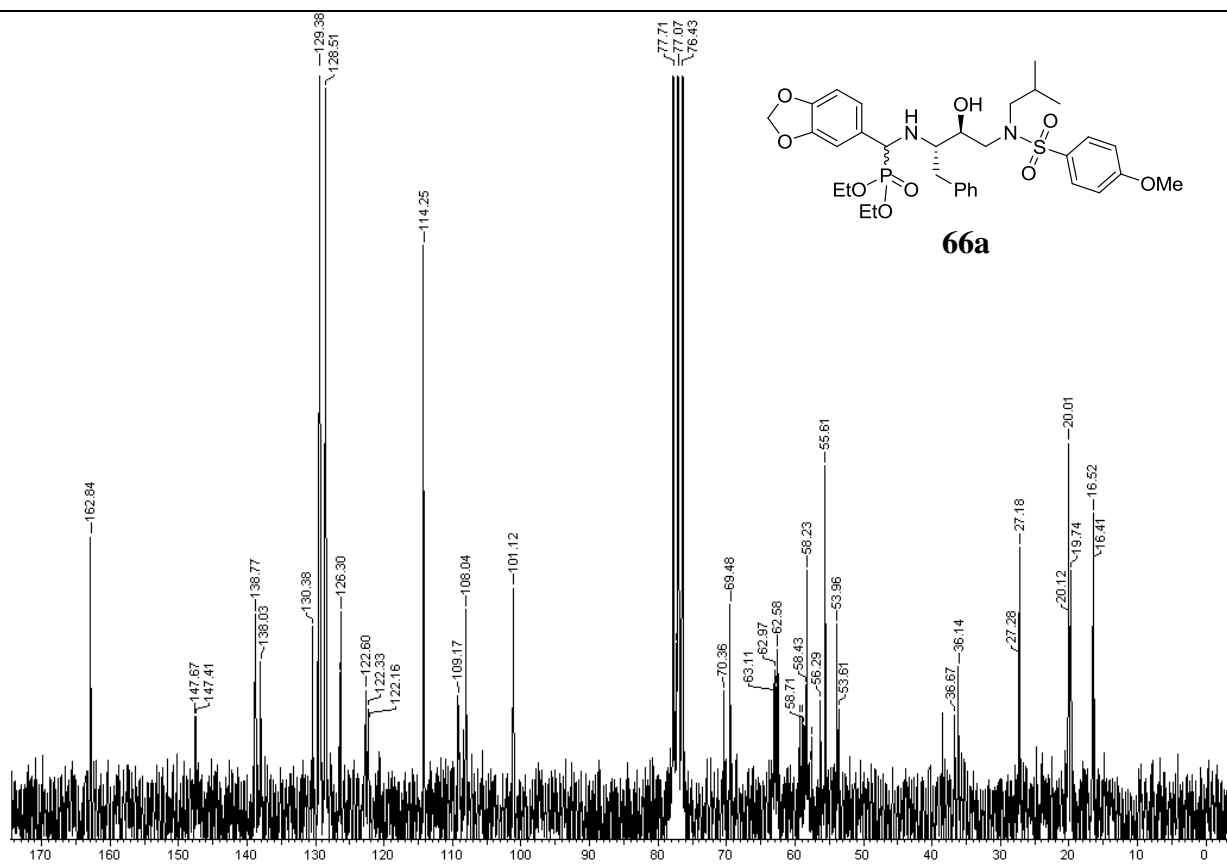
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) of **40a**<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) of **40a**

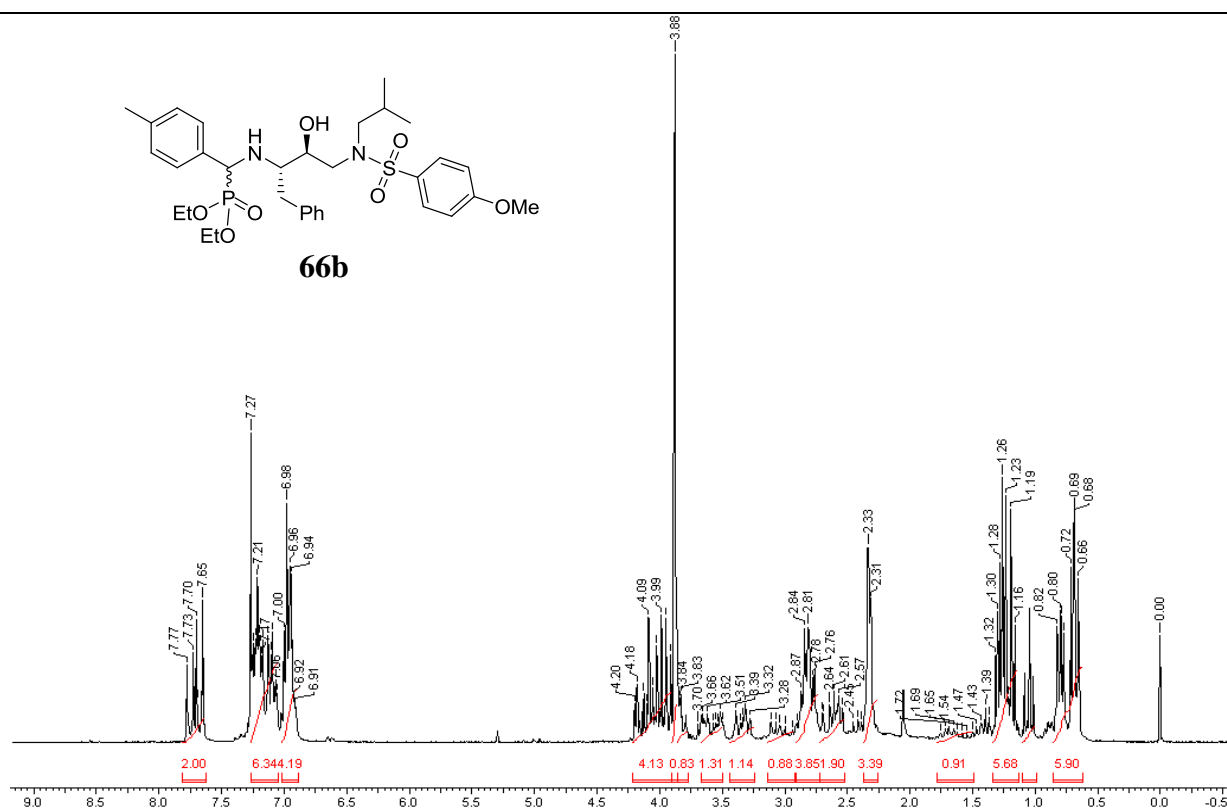
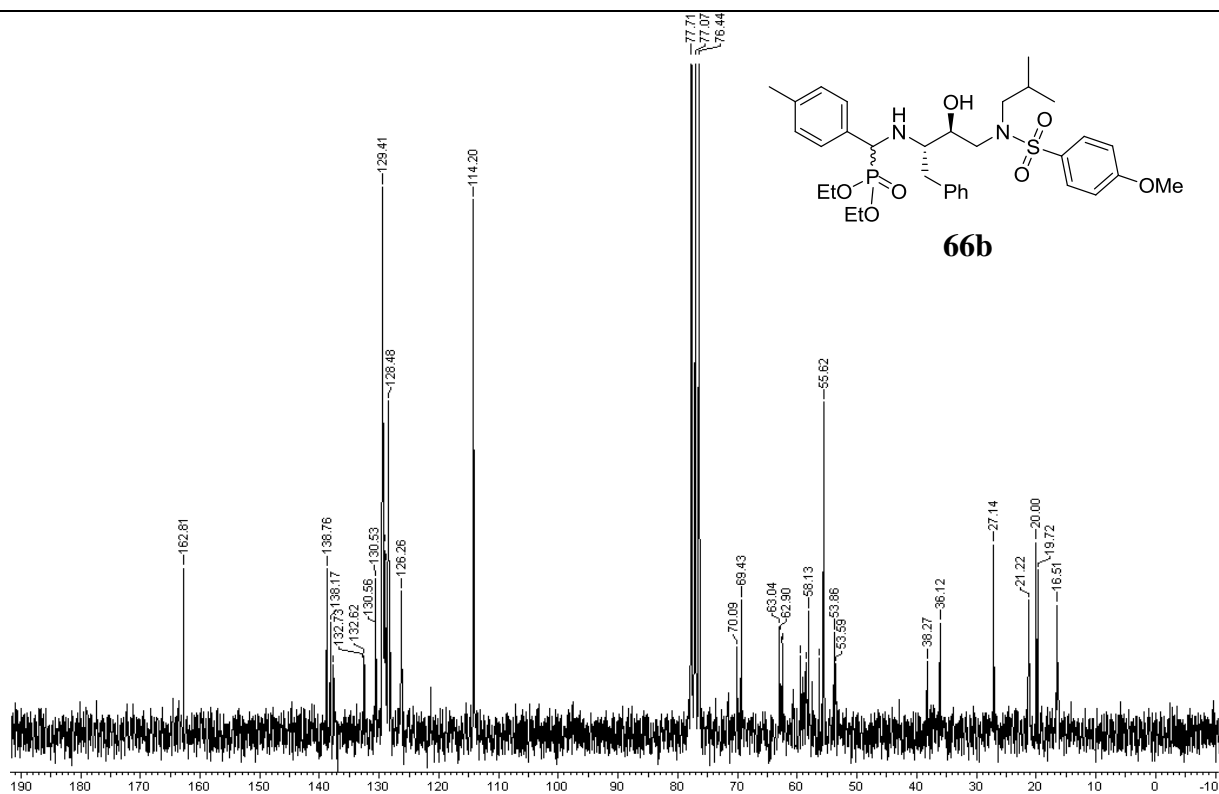
<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **40b**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **40b**

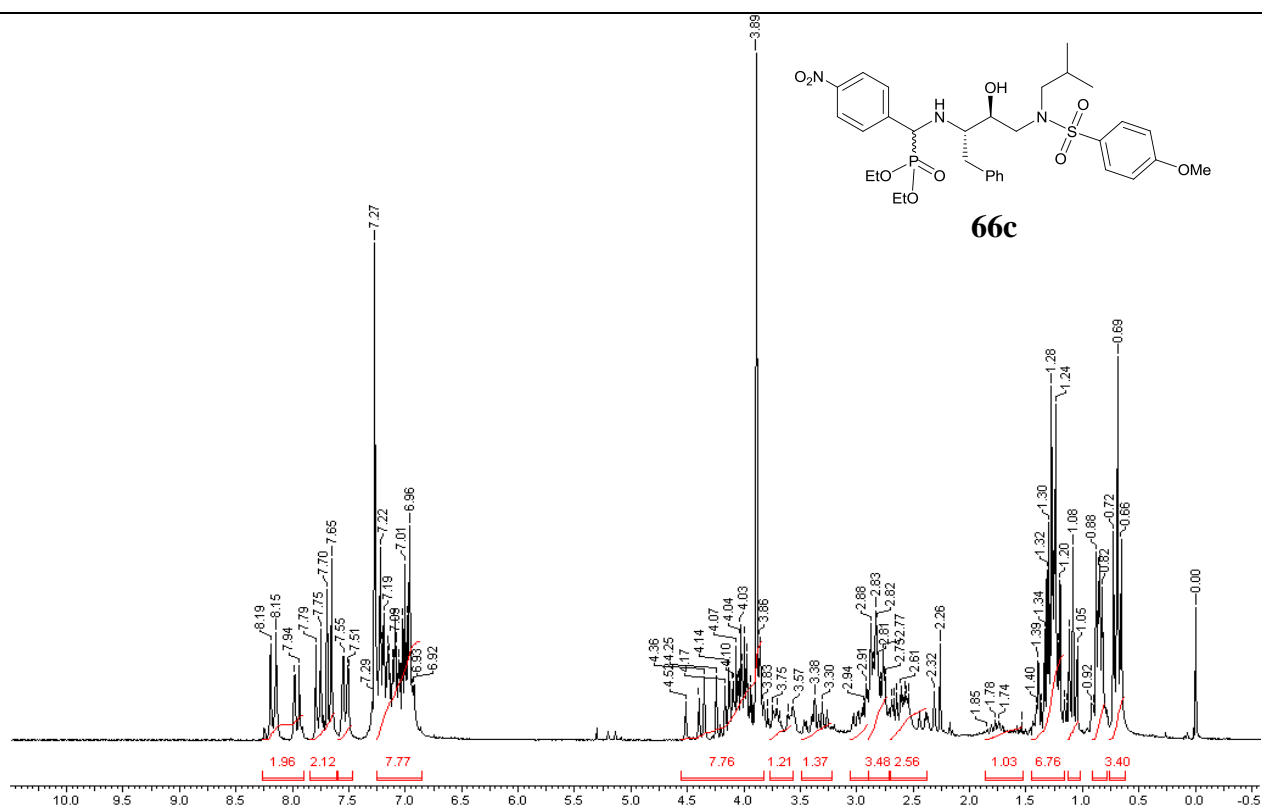
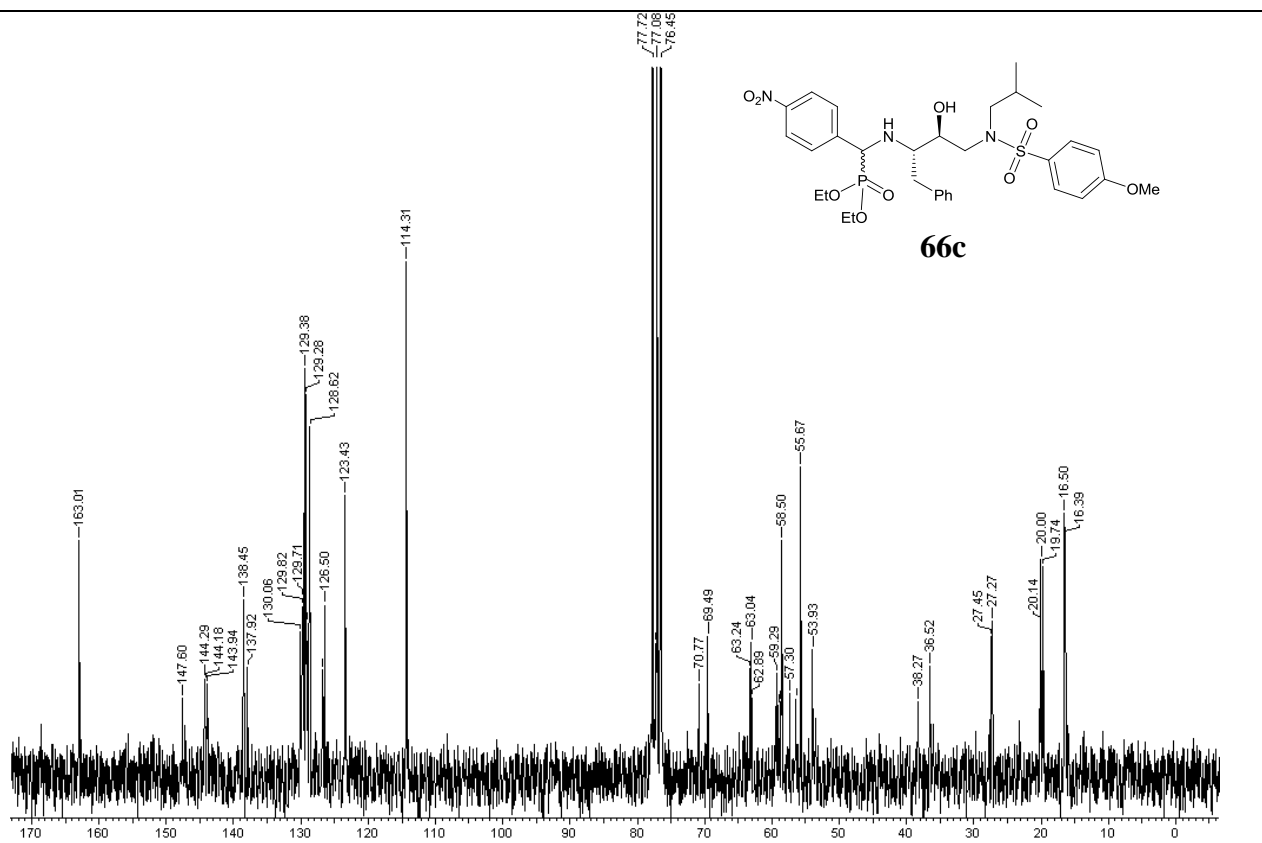
$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) of **40c** $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) of **40c**

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) of **40d**<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) of **40d**

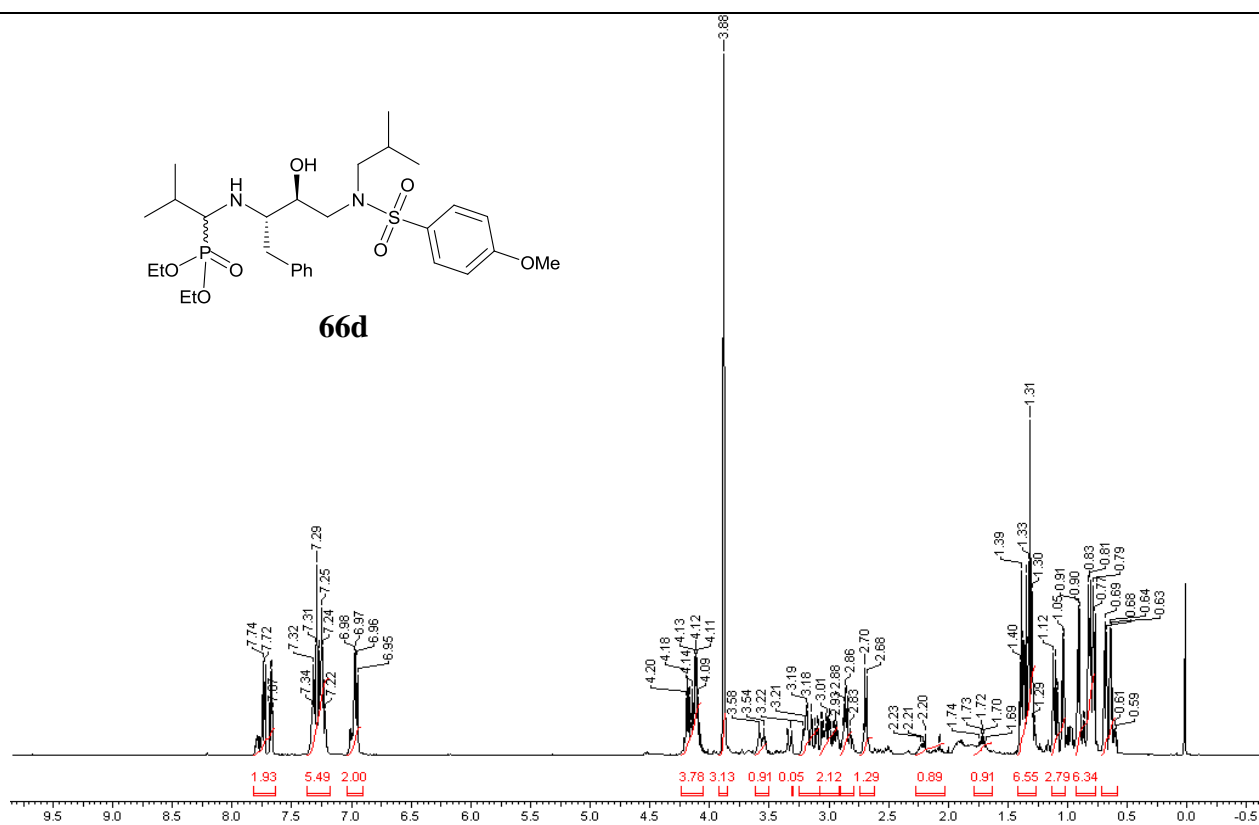
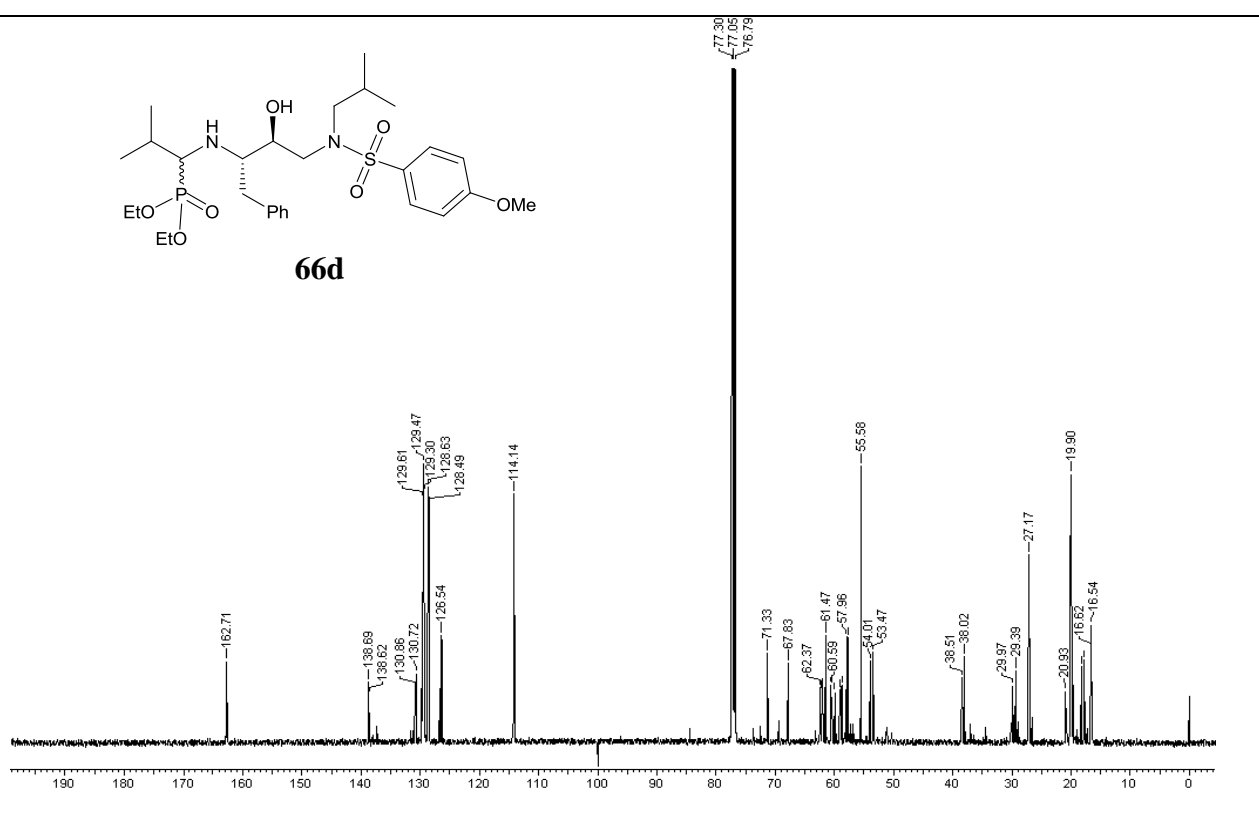
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **40e** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **40e**

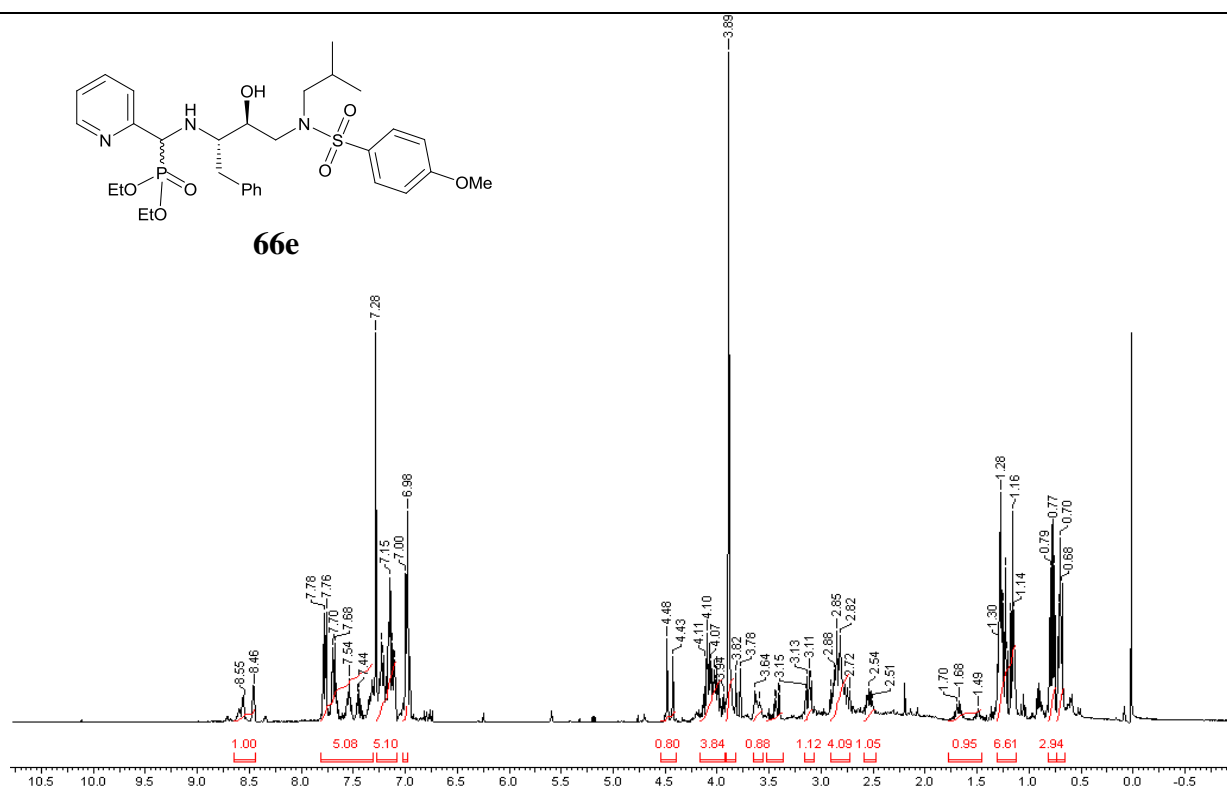
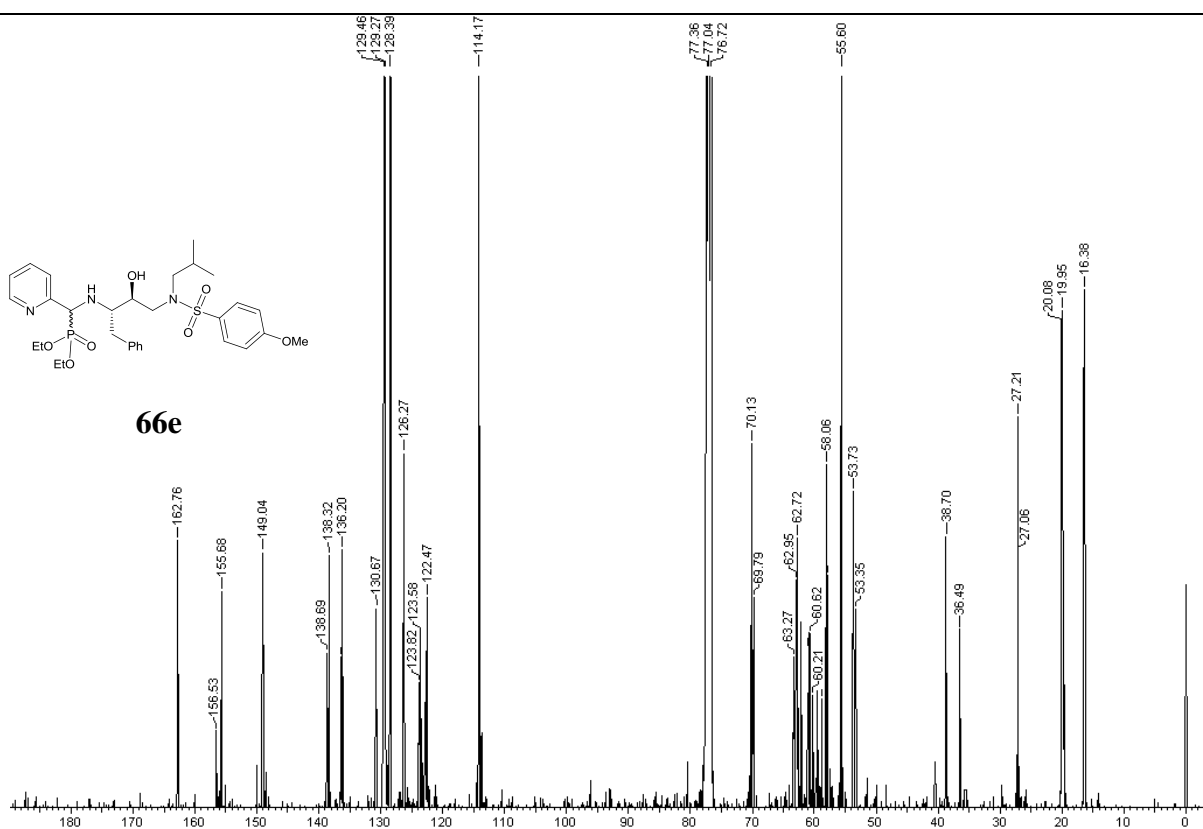
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) of **66a** $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) of **66a**

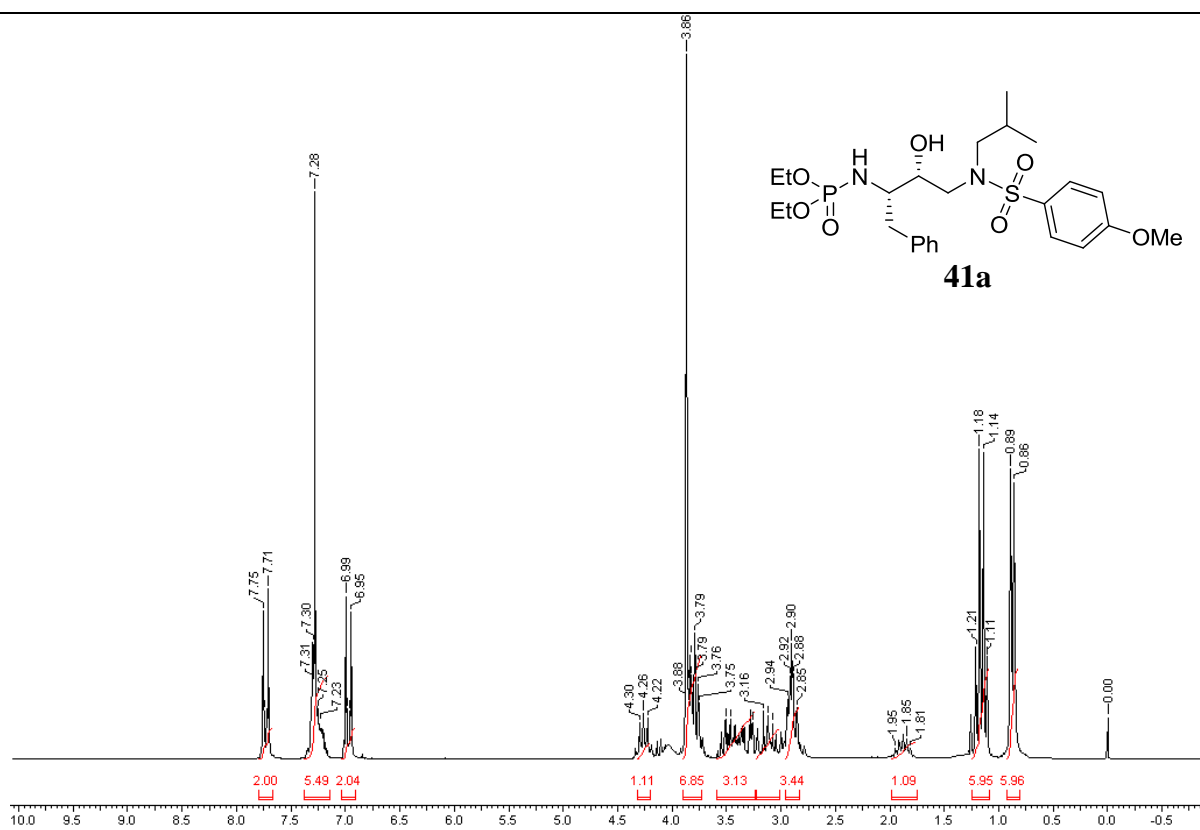
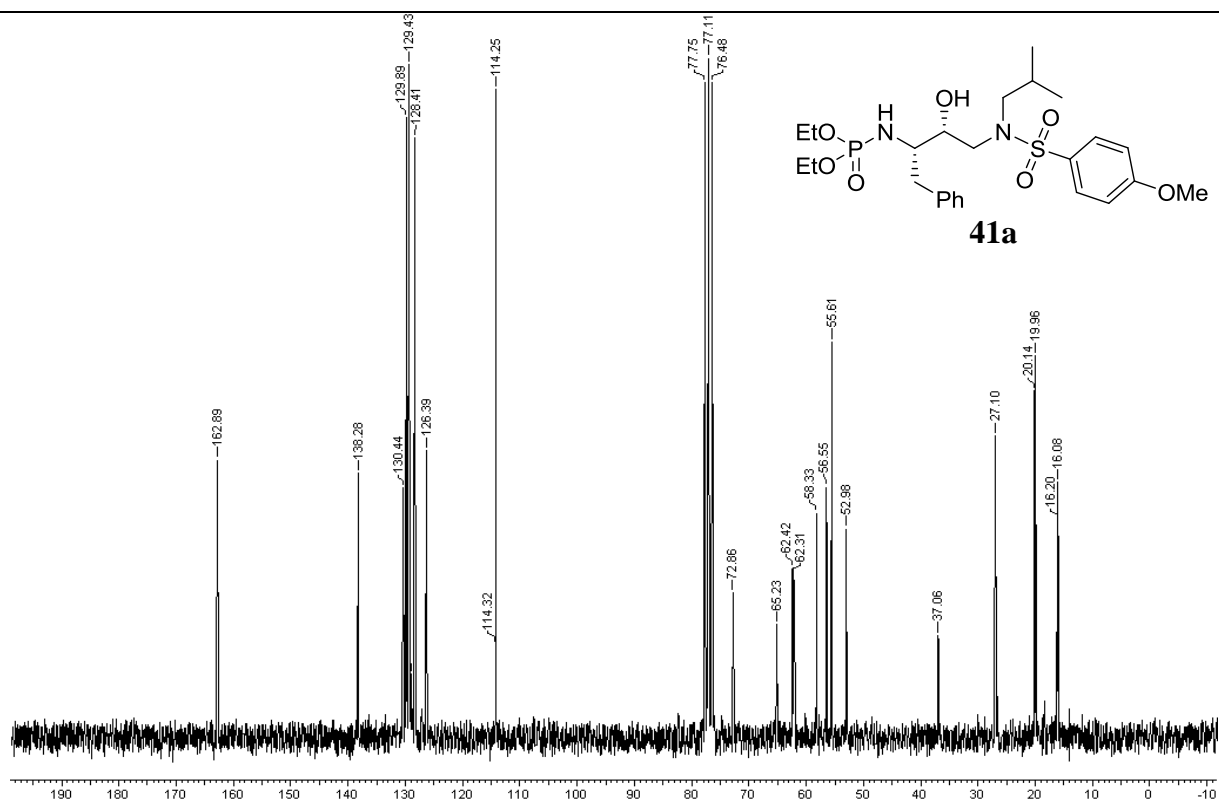
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **66b** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **66b**

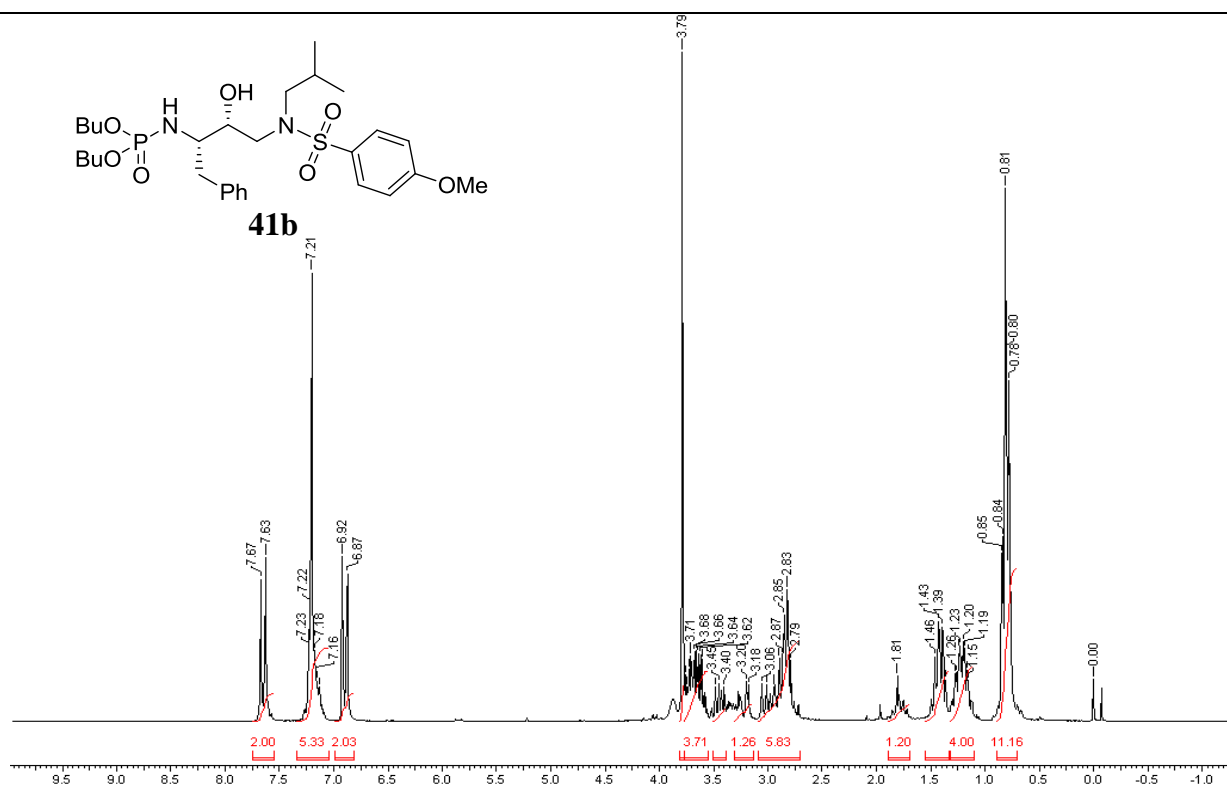
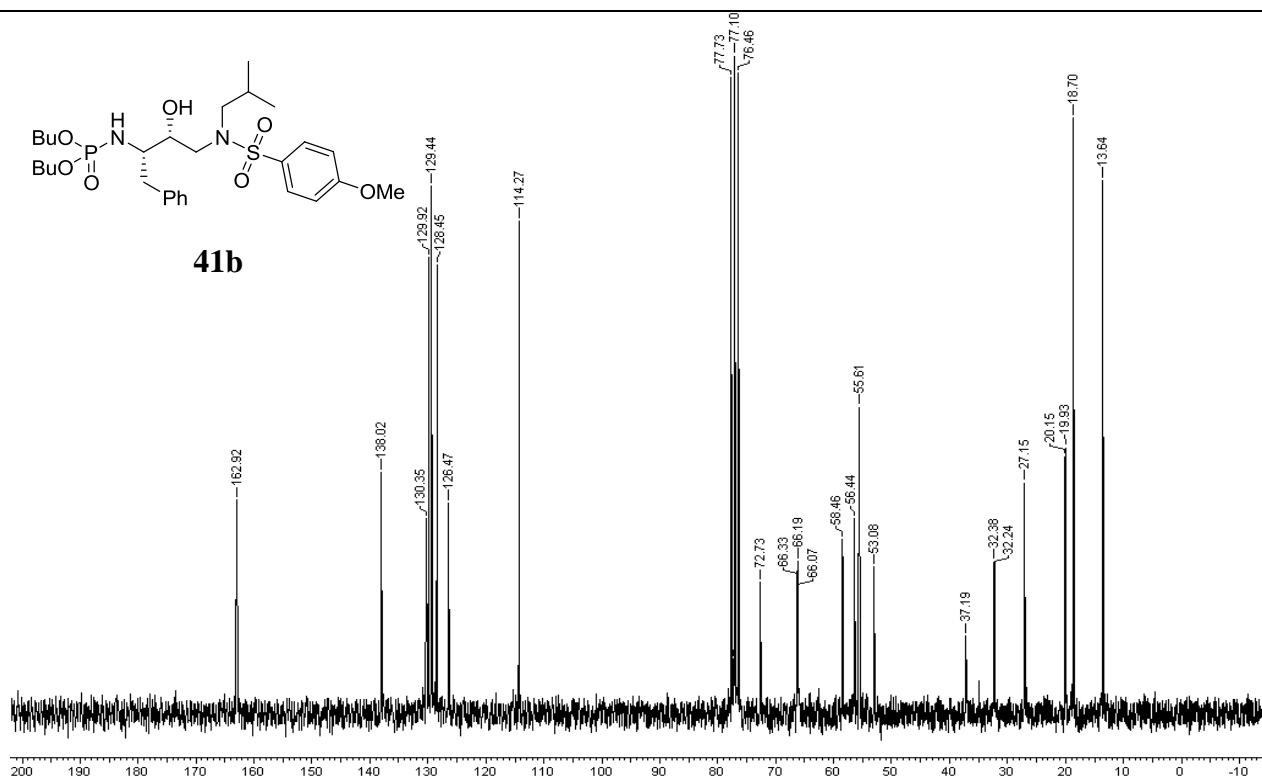
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **66c** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **66c**



$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) of **66d** $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ) of **66d**

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) of **66e** $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) of **66e**

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **41a** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **41a**

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **41b** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **41b**

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## **Chapter 3**

### **Design and Synthesis of Cysteine Protease Inhibitors**

#### **Section A**

#### **Synthesis of Vinylaminophosphonates as Cysteine Protease Inhibitors**

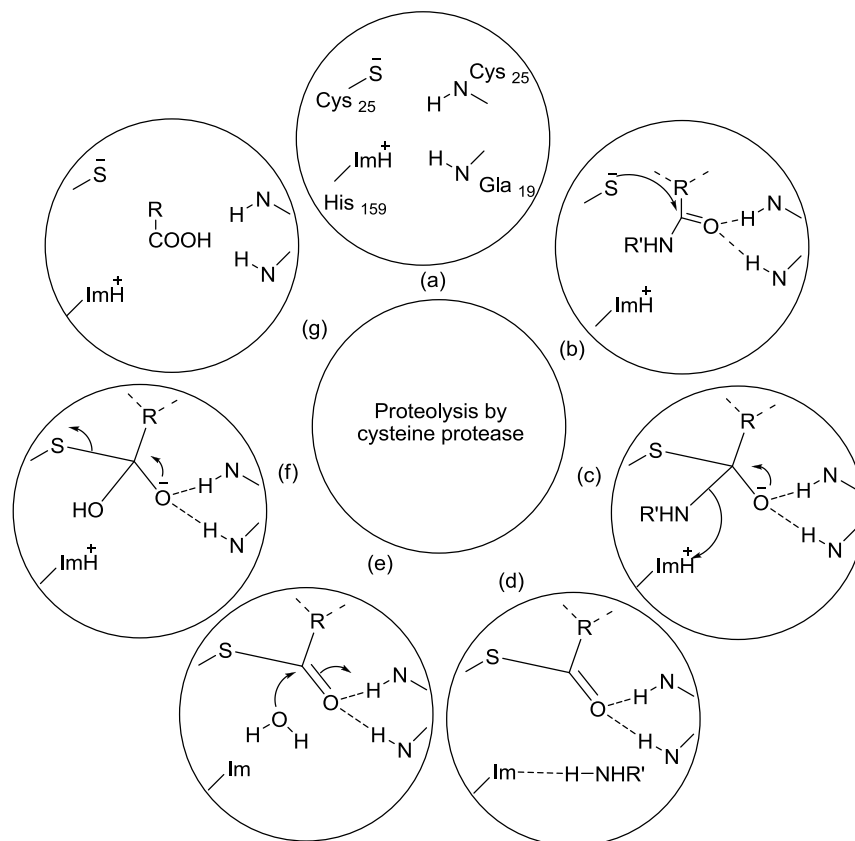
## Introduction

Cysteine proteases are sulfhydryl protease which catalyzes the hydrolysis of peptide, amide, ester and thiol ester bonds. Many normal physiological functions necessitate cysteine protease *e.g.* cathepsins are involved in protein breakdown in lysosomes, antigen presentation, proteolytic processing of proenzymes and prohormones, fertilization, cell proliferation, differentiation and apoptosis etc. Disharmony in the activity of cysteine protease may lead to various pathologies such as rheumatoid arthritis, multiple sclerosis, neurological disorders, tumours and osteoporosis.<sup>1</sup> Therefore, cathepsins could be potential drug targets for treating systemic human diseases such as asthma, rheumatoid arthritis, cancer, osteoporosis and atherosclerosis.<sup>2</sup> Calpains have also been involved in several diseases such as type 2 diabetes, traumatic brain, muscular dystrophy, cerebral ischemia, Alzheimer's disease, multiple sclerosis and spinal cord injury.<sup>3</sup> Various cysteine proteases are implicated in variety of pathogenic conditions or disorders such as gingipain in gingivitis,<sup>4</sup> caspases in neurodegenerative disorders, separase in cancer,<sup>5</sup> falcipain in malaria, legumain in parasitic infections<sup>6</sup> and clostripain in bacterial infections. Cathepsin B and Calpain I are also believed to be involved in apoptosis.<sup>7</sup> A molecule that can prevent the function of a protease is known as a protease inhibitor. Therefore, study directed towards design and synthesis of cysteine protease inhibitors has gained considerable importance in the field of medicinal chemistry for the development of new candidate drugs.<sup>1</sup>

## Mechanism of Proteolysis by Cysteine Protease

The general mechanism of cysteine protease action has been very well studied,<sup>8</sup> with papain as the model enzyme. Imidazole of the histidine (His) polarizes the thiol of the cysteine (Cys) thus allowing for deprotonation (Figure 1a). The substrate then enters the active site and gets non-covalently bounded *via* hydrogen bonds of the carbonyl groups forming the enzyme-substrate complex (Figure 1b). Then attack of Cys thiol to the carbonyl group allows formation of a tetrahedral intermediate (Figure 1c). Protonation of the leaving group takes place by the imidazole of the catalytic His which act as a general acid, resulting in the acyl

intermediate (Figure 1d). Followed by the abstraction of a proton from a water molecule by catalytic His (Figure 1e). The activated water molecule then hydrolyzes the thioester of the acyl intermediate bond (Figure 1f) resulting in the formation of free acid (Figure 1g) and enzyme active site gets free for another hydrolysis reaction (Figure 1a).

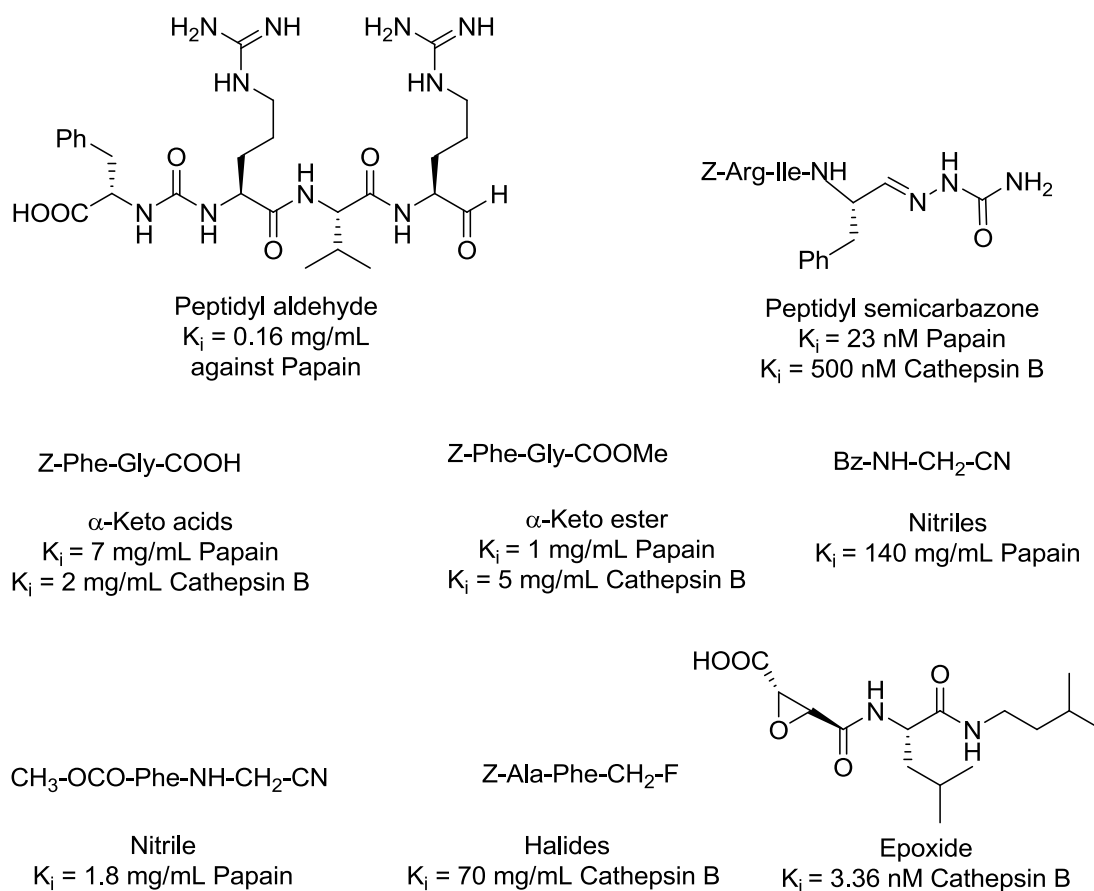


**Figure 1.** Proteolysis by cysteine proteases (adapted from Ref. 1b).

### Cysteine Protease Inhibitors

The molecules that cease or slow down the proteolytic activity of the cysteine protease are known as cysteine protease inhibitors. Based on the mechanism many inhibitors have been designed, synthesized and evaluated for the inhibition of cysteine proteases.<sup>1,9</sup> Most of the inhibitor follow the usual structural scheme for development of protease inhibitors. This comprises a peptide segment for the recognition of the enzyme and an electrophilic group which can react with the cysteine residue of the active site. Many cysteine protease inhibitors have been designed and synthesized by

various research groups<sup>1</sup> all over the world which includes peptidyl aldehyde, semicarbazone,  $\alpha$ -keto acids/esters, nitriles, halides and epoxides (Figure 2).

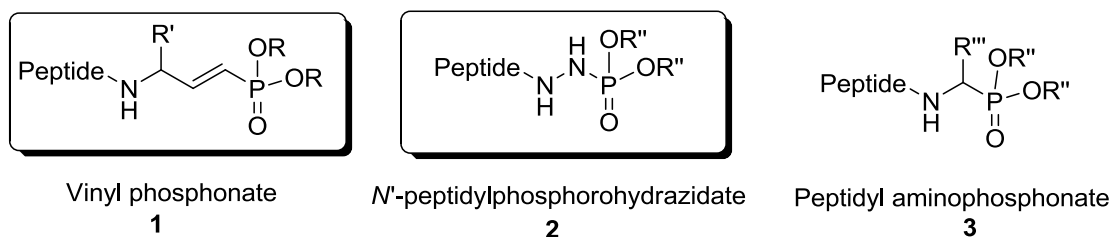


**Figure 2.** Known cysteine protease inhibitors.

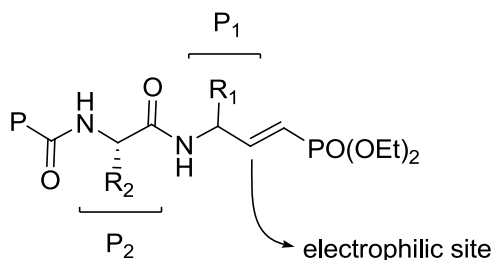
## Present Work

Based on the mechanism of proteolysis by cysteine protease, we have designed three classes of compounds *viz.* peptidyl-vinylphosphonate **1** and *N'*-peptidylphosphorohydrazidate **2** as shown in Figure 3. The designed molecules contain phosphonates or vinylic phosphonate group as electrophilic centre required for the nucleophilic attack of the cysteine thiol. The peptidyl character of the designed molecule is required for the hydrophobic interaction in the active site of the enzyme (Figure 4).





**Figure 3.** Designed cysteine protease inhibitors.



**Figure 4.** Designing of the cysteine protease inhibitors.

The peptidyl aminophosphonates **3** had already been studied<sup>10</sup> and are found to be inhibitors of serine protease, while peptidyl-vinylphosphonate **1** and *N'*-peptidylphosphorohydrazidate **2** have not been studied so far for their inhibition of cysteine proteases.

The present work describes design, synthesis and enzyme inhibition studies of peptidyl-vinylphosphonate **1** and *N'*-peptidylphosphorohydrazidate **2**.

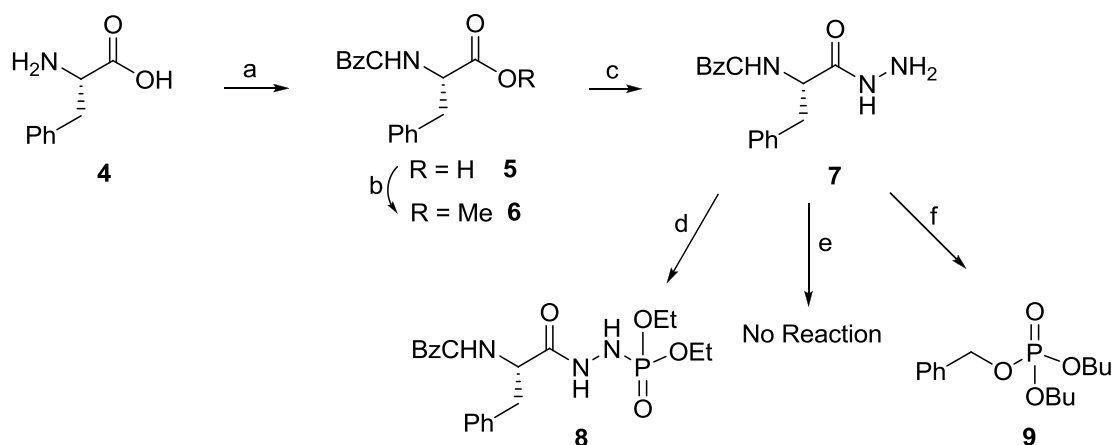
## Results and Discussion

### Synthesis of *N'*-peptidylphosphorohydrazidate

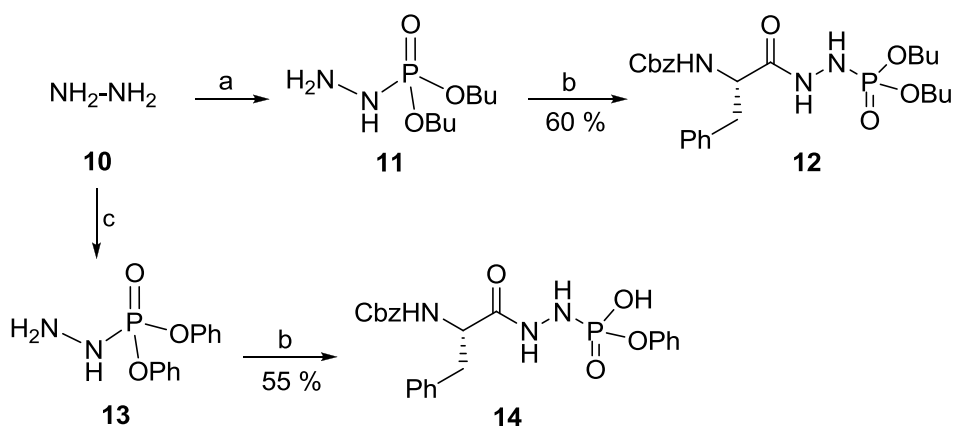
The synthesis of *N'*-peptidylphosphorohydrazidates **8**, **12** and **14** were accomplished starting from protected phenyl alanine (Scheme 1) or hydrazine hydrate (Scheme 2). For the synthesis of *N'*-peptidylphosphorohydrazidates **8**, the acid and amine functional groups of phenyl alanine **4** was protected with methyl ester and benzyl carbamate, respectively to obtain compound **6**. Compound **6** was treated with excess of hydrazine hydrate to afford protected phenylalanine hydrazide **7** in 96% yields. Thereafter, Atherton-Todd<sup>11</sup> reaction was performed on compound **7** with diethylphosphite to furnish the desired diethyl *N'*-peptidylphosphorohydrazidate **8**.

However, in case of dibutyl phosphite, Atherton-Todd<sup>11</sup> reaction on compound **7** did not work under the similar reaction conditions. This could be attributed to the steric hindrance of bulkier groups of dibutyl phosphite. Therefore, strong base *e.g.* aq. NaOH was used for completion of the same reaction instead and to our surprise major by-product phosphate **9** was obtained. The formation of product **9** could be due to the fact that aq. NaOH is a strong base, hence first deprotection of the benzyl carbamate functionality takes place to form free benzyl alcohol which subsequently underwent Atherton-Todd reaction under the same reaction conditions to furnish compound **9**.

Therefore, we changed our synthetic strategy to accomplish the synthesis of compounds **12** and **14**. We opined that Atherton-Todd reaction on hydrazine hydrate followed by peptide coupling reaction would be an ideal alternative (Scheme 2). Following this strategy, compound **11** was obtained by the reaction of dibutyl phosphite and hydrazine, which was followed by peptide coupling reaction with carbamate protected phenylalanine to furnish one of the desired compound **12**. Similar set of reaction sequences led us to compound **14**.



**Scheme 1.** Reagents and conditions: (a) CBz-Cl, aq. NaOH, 88%; (b) MeOH, HCl, reflux, 2 h, 93 %; (c) NH<sub>2</sub>-NH<sub>2</sub>, MeOH, rt, 1 h, 96%; (d) CCl<sub>4</sub>, diethyl phosphite, DCM, K<sub>2</sub>CO<sub>3</sub>, TBAB, 12 h, 67%; (e) CCl<sub>4</sub>, dibutyl phosphite, DCM, K<sub>2</sub>CO<sub>3</sub>, TBAB; (f) CCl<sub>4</sub>, dibutyl phosphite, DCM, aq. NaOH, 58%.



**Scheme 2.** Reagents and conditions: (a)  $\text{CCl}_4$ , Dibutyl phosphite, DCM,  $\text{K}_2\text{CO}_3$ , TBAB, 12 h, 82%; (b) **5**, DCC, HOBT, THF, 12 h; (c)  $\text{CCl}_4$ , Diphenyl phosphite, DCM,  $\text{K}_2\text{CO}_3$ , TBAB, 3 h, 80%.

### *In Vitro* Protease Inhibition Activity of *N'*-Peptidylphosphorohydrazidate

The synthesized *N'*-peptidylphosphorohydrazidates **8**, **12** and **14** were tested for their *in vitro* protease inhibition activities against papain and the results are summarized in Table 4. The  $\text{IC}_{50}$  value of the compound **8** was calculated to be 600  $\mu\text{M}$ .

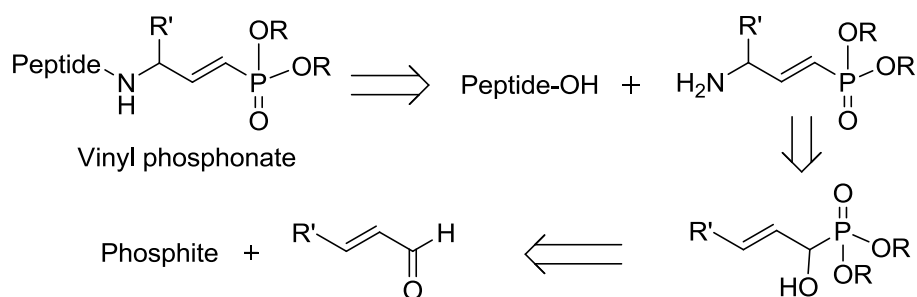
**Table 1.** Protease inhibition activity of *N'*-peptidylphosphorohydrazidate.

Sl. No.	Compound	Molarity Used ( $\mu\text{M}$ )	Inhibition Activity (%)
1	<b>8</b>	1200	83 ( $\text{IC}_{50}$ 600 $\mu\text{M}$ )
2	<b>12</b>	500	3
3	<b>14</b>	500	1

The result revealed that except compound **8**, *N'*-peptidylphosphorohydrazidate derivatives **12** and **14** did not inhibit the model enzyme papain. However, only compound **8** showed low level of inhibition against papain with  $\text{IC}_{50}$  value of 600  $\mu\text{M}$ . Therefore, further exploration of *N'*-peptidylphosphorohydrazidate derivatives as protease inhibitors was not carried out and instead it was decided to study peptidyl-vinylphosphonate as protease inhibitors.

## Synthesis of Peptidyl-Vinylphosphonate

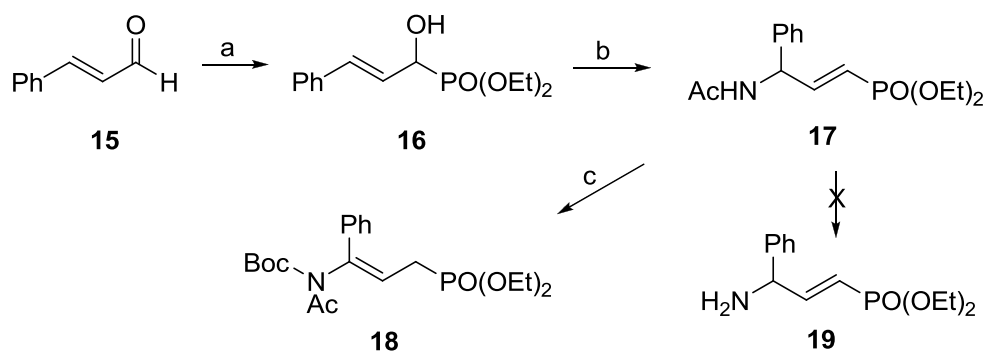
The synthesis of peptidyl-vinylphosphonates can be achieved as depicted in retrosynthetic Scheme 3, key step being conversion of the hydroxyphosphonate to  $\gamma$ -amino-vinylphosphonate. The conversion of hydroxyphosphonate to  $\gamma$ -amino-vinylphosphonate in turn could be achieved either by Ritter reaction<sup>12</sup> (Approach I) or Tsuji-Trost reaction<sup>13</sup> (Approach II).



**Scheme 3.** Retrosynthesis of peptidyl-vinylphosphonates.

### Approach I: Ritter Reaction

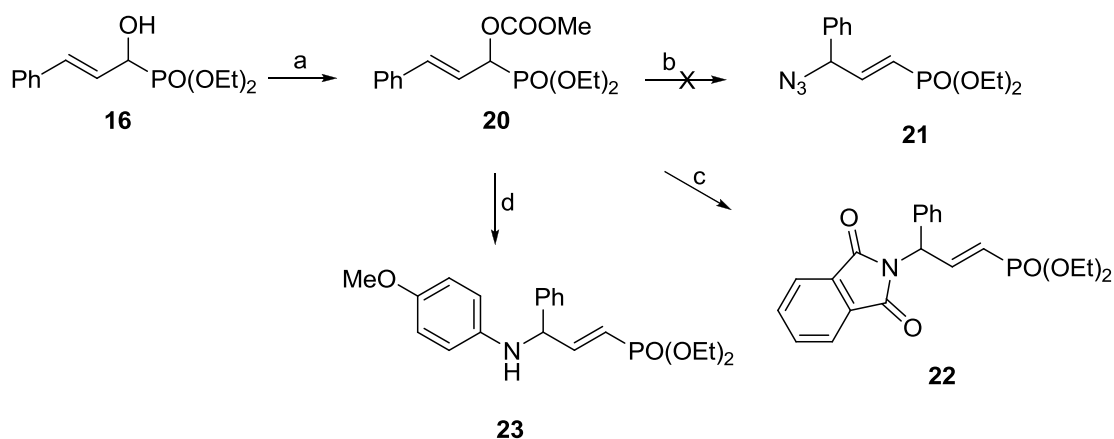
In our attempt to synthesise  $\gamma$ -amino-vinylphosphonate via the Ritter reaction, cinnamaldehyde **15** was subjected to the hydrophosphinylation reaction in presence of a base (triethyl amine, TEA) to form hydroxyphosphonate **16** (Scheme 4). When hydroxyphosphonate **16** was subjected to the Ritter reaction in presence of Lewis acid ( $\text{BF}_3 \cdot \text{Et}_2\text{O}$ ) and acetonitrile as solvent at room temperature for overnight,  $\gamma$ -acetamino-vinylphosphonates **17** was obtained exclusively. However, the deprotection of the acetate group of the acetamide **17** was found to be very difficult. In the literature,<sup>14</sup> it has been reported that deprotection of the acetate group of acetamide could be achieved by following a three steps protocol *i.e.* Boc protection followed by acetate deprotection and Boc deprotection. However, in our case the very first step *i.e.* Boc protection resulted in the double bond migration to furnish product **18**. Since other reported<sup>15</sup> methods of deprotection of the acetate group required harsh reaction conditions which may result in the hydrolysis of the phosphonate ester, therefore, the Ritter reaction approach was not pursued further and instead alternative Tsuji-Trost reaction was attempted.



**Scheme 4.** Reagents and conditions: (a) DEP, TEA, 0°C-rt, 3 h, 77%; (b)  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ , MeCN, 0° C-rt 24 h, 70%; (c)  $\text{Boc}_2\text{O}$ , DMP, MeCN, rt, 12 h, 83%.

### Approach II: Tsuji-Trost Reaction

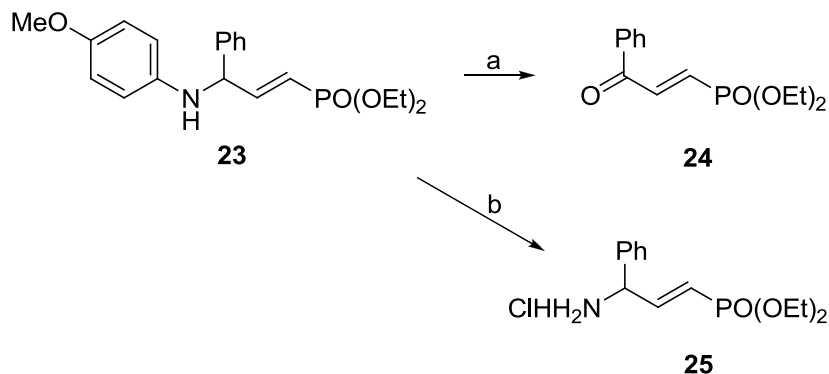
In this approach, first the hydroxyl group of compound **16** was transformed into carbonate derivative **20** (Scheme 5). The carbonate **20** was then subjected to Tsuji-Trost reaction conditions with various nucleophiles like sodium azide, potassium phthalimide and *p*-anisidine. No corresponding product formation was observed when carbonate **20** was reacted with sodium azide. In case of Tsuji-Trost reaction with potassium phthalimide, corresponding product **22** was obtained albeit in low yield. However, the Tsuji-Trost reaction of **20** with *p*-anisidine was satisfactory furnishing the corresponding product **23** in excellent yield.



**Scheme 5.** Reagents and conditions: (a)  $\text{MeOCOCi}$ , Py, MeCN, 0°C-rt, 12 h, 70%; (b)  $\text{NaN}_3$ , THF:H<sub>2</sub>O,  $\text{Pd}(\text{OAc})_2$ ,  $\text{PPh}_3$  rt/reflux; (c) K-phthalimide, DMF,  $\text{Pd}(\text{OAc})_2$ ,  $\text{PPh}_3$ , 80°C, 3 h, 33%; (d) *p*-anisidine, THF,  $\text{Pd}(\text{OAc})_2$ ,  $\text{PPh}_3$ , rt, 1 h, 95%.

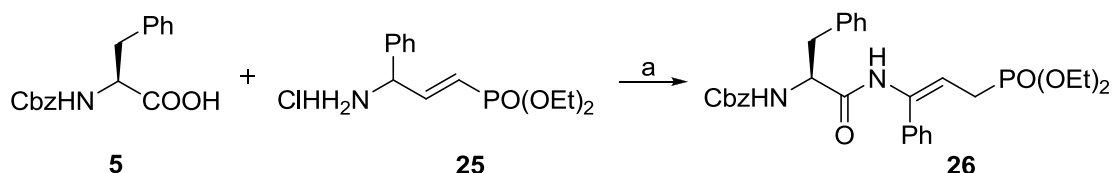
The deprotection of the PMP group was attempted using  $\text{CAN}^{16}$  however, by-product **24** was obtained in low yield. The deprotection of PMP group in compound

**23** was finally achieved by reaction with trichloroisocyanuric acid (TCCA)<sup>17</sup> in acidic medium to furnish free  $\gamma$ -amino-vinylphosphonate **25** which was obtained as its hydrochloride salt due to acidic work-up (HCl/ethyl acetate).



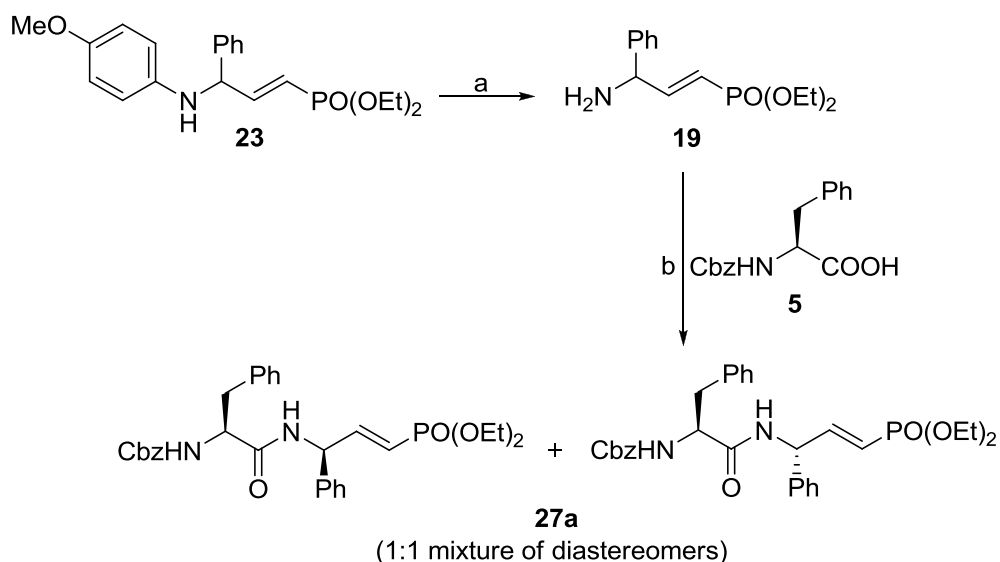
**Scheme 6.** Reagents and conditions: a) CAN, MeCN:H<sub>2</sub>O, rt, 10%; b) TCCA, MeCN:H<sub>2</sub>O, 1 M H<sub>2</sub>SO<sub>4</sub> 12 h then HCl, 57%.

The salt **25** was subjected to peptide coupling reaction with Cbz protected phenylalanine using DCC/HOBt as coupling reagent<sup>18</sup> to furnish a product **26** which was found to be allylic phosphonate by its spectral data instead of the expected vinylic phosphonate (Scheme 7). The formation of allylic phosphonate **26** could be due to the use of triethylamine as base which resulted in the isomerisation of the double bond. Triethyl amine was used to form *in situ* free amine from the hydrochloride salt **25**.



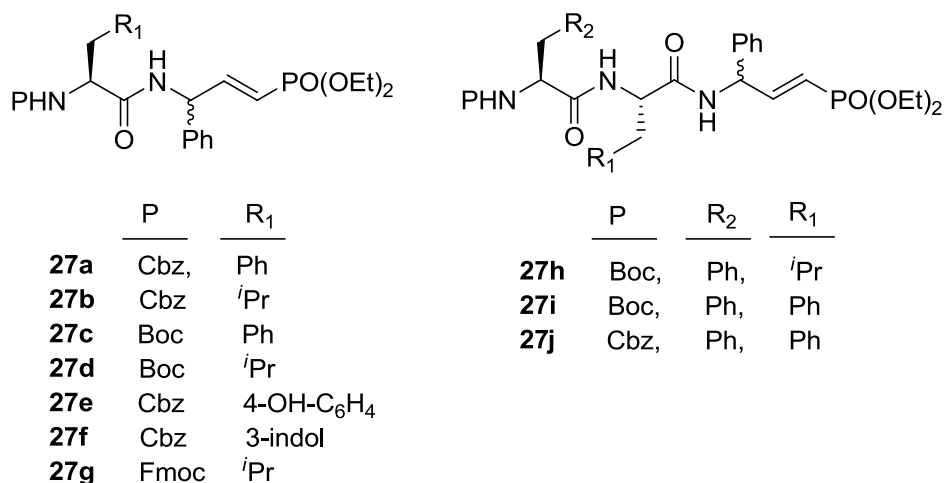
**Scheme 7.** Reagents and conditions: (a) Vinylaminophosphonate **25**, Et<sub>3</sub>N, DCC, HOBt, THF 0°C-rt, 52%.

Since coupling reaction of hydrochloride salt **25** resulted in isomerisation of double bond therefore, we opined that deprotection of PMP group can be carried out using the earlier used deprotection condition *i.e.* with TCCA however, no acidic work up will be carried out to get the free amine **19** (Scheme 8). Afterwards, compound **19** was subjected to peptide coupling reaction with benzyl carbamate protected phenylalanine to furnish peptidyl-vinylaminophosphonate **27a** as an inseparable mixture of diastereomers (1:1).<sup>19</sup>



**Scheme 8.** Reagents and conditions: (a) TCCA, MeCN:H<sub>2</sub>O, 1 M H<sub>2</sub>SO<sub>4</sub>, 12 h, 59%; (b) DCC, HOBT, THF 0°C-rt, 53%.

Similar peptide coupling reactions were carried out with various protected amino acids and dipeptides to furnish peptidyl-vinylaminophosphonate **27a-j** as shown in Figure 5. All these peptidyl-vinylaminophosphonate **27a-j** were isolated as a mixture of diastereomers in the ratio of 1:1 and our all attempts<sup>19</sup> to separate these diastereomers by using different chromatography techniques were unsuccessful.



**Figure 5.** Peptidyl vinylaminophosphonate synthesized as mixture of diastereomers (1:1).

***In Vitro* Protease Inhibition Activity of Peptidyl Vinylaminophosphonate**

The synthesized peptidyl vinylaminophosphonates **27a-j** were tested for their *in vitro* protease inhibition activity against papain and the IC<sub>50</sub> values are compiled in Table 3.

**Table 3.** *In vitro* protease inhibition activity of peptidyl vinylaminophosphonates.

Entry	Compound	IC <sub>50</sub> ( $\mu$ M)	Entry	Compound	IC <sub>50</sub> ( $\mu$ M)
1	<b>27a (Cbz-Phe-Vp)</b>	30	6	<b>27f (Cbz-Trp-Vp)</b>	> 200
2	<b>27b (Cbz-Leu-Vp)</b>	>200	7	<b>27g (Fmoc-Leu-Vp)</b>	>200
3	<b>27c (Boc-Phe-Vp)</b>	40	8	<b>27h (Boc-Phe-Leu-vp)</b>	132
4	<b>27d (Boc-Leu-Vp)</b>	>200	9	<b>27i (Boc-Phe-Phe-Vp)</b>	83
5	<b>27e (Cbz-Tyr-Vp)</b>	54	10	<b>27j (Cbz-Phe-Phe-Vp)</b>	125

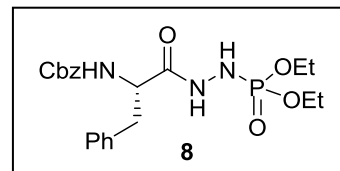
The activity data revealed that peptidyl-vinylaminophosphonates having phenylalanine in P<sub>2</sub> pocket (compounds **27a** and **27c**) were found to be more active than peptidyl-vinylaminophosphonates with leucine and other amino acids in P<sub>2</sub> pocket (compounds **27b**, **27d**, **27e** and **27f**). However, it is interesting to note that dipeptidyl-vinylaminophosphonates (**27a-g**) were more active than their corresponding tripeptidyl-vinylaminophosphonates (**27h-j**).



## Experimental

### (S)-Diethyl N'-2-(2-oxo-2-phenylethylideneamino)-3-phenylpropanoyl-phosphoro-hydrazidate (**8**)

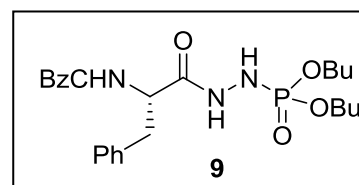
To a stirred solution of hydrazine **7** (313 mg, 1 mmol) in CCl<sub>4</sub> (0.8 mL) and DCM (1.6 mL), K<sub>2</sub>CO<sub>3</sub> (200 mg, 1.4 mmol) and TBAB (10 mg) were added and the reaction mixture was stirred for 15 min at rt. Diethyl phosphite



was added to the reaction mixture and the resulting mixture was further allowed to stir overnight at rt. After completion of the reaction (TLC), the reaction mixture was filtered and the residue was washed with DCM (25 mL), concentrated under vacuum to obtain crude product which was purified by column chromatography using petroleum ether and ethyl acetate (1:1) as eluant to obtain pure product **8** (300 mg, 67%) as colourless syrup;  $[\alpha]_D^{20} = -9.40$  (*c* 1.0, MeOH); IR (CHCl<sub>3</sub>): 3283, 3208, 3018, 1691, 1605, 1542, 1496, 1265, 1216 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  1.27 (t, <sup>3</sup>J<sub>PC</sub> = 7.2 Hz, 3H), 1.38 (t, <sup>3</sup>J<sub>PC</sub> = 7.7 Hz, 3H), 3.00-3.17 (m, 2H), 4.02-4.33 (m, 4H), 4.45-4.64 (m, 1H), 4.97-5.03 (m, 2H), 7.15-7.33 (m, 10H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  16.0 (d, <sup>3</sup>J<sub>PC</sub> = 3.3 Hz), 16.1 (d, <sup>3</sup>J<sub>PC</sub> = 3.3 Hz), 38.5, 54.6, 65.2 (d, <sup>2</sup>J<sub>PC</sub> = 5.4 Hz), 63.7 (d, <sup>2</sup>J<sub>PC</sub> = 5.4 Hz), 67.1, 126.9, 127.0, 127.9, 128.0, 128.2, 128.5, 128.6, 129.4, 129.6, 136.1, 136.1, 156.0, 171.1; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>):  $\delta$  4.20; (ESI): *m/z* 450.9 (M+H)<sup>+</sup>, 472.9 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>21</sub>H<sub>28</sub>N<sub>3</sub>O<sub>6</sub>P: C, 56.12; H, 6.28; N, 9.35. Found: C, 56.24; H, 6.36; N, 9.29.

### (S)-Dibutyl N'-2-(2-oxo-2-phenylethylideneamino)-3-phenylpropanoyl-phosphoro-hydrazidate (**12**).

To the stirred solution of acid **5** (299 mg, 1 mmol), hydrazine **11** (268 mg, 1.2 mmol) and HOBt (340 mg, 2.2 mmol) in THF (2.5 mL), DCC (230 mg, 1.1 mmol) was added at 0°C. The reaction mixture was allowed to

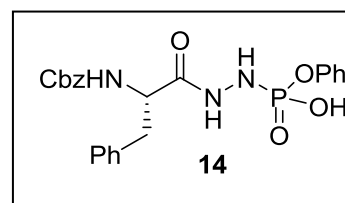


stir at 0°C for 2 h and at rt for further 12 h. After completion of the reaction (TLC), the reaction mixture was filtered and concentrated in vacuum. The crude was redissolved in ethyl acetate (50 mL), washed with water (3x20 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to obtain the crude product which was purified by silica gel

column chromatography using ethyl acetate: petroleum ether (1:1) as eluant to yield the pure product **9** (300 mg, 60%) as colourless syrup;  $[\alpha]_D^{20} = -1.26$  (*c* 1.2, MeOH); IR (CHCl<sub>3</sub>): 3285, 3210, 3020, 2967, 1690, 1605, 1542, 1387, 1215 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  0.88-0.97 (m, 6H), 1.36-1.42 (m, 4H), 1.59-5.66 (m, 4H), 2.61-3.19 (m, 2H), 3.34-3.35 (m, 1H), 4.06-4.12 (m, 4H), 5.01 (s, 2H), 7.44-7.88 (m, 10H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  14.1, 19.8, 33.4, 33.5, 39.4, 56.4, 67.6, 68.3 (d, <sup>2</sup>J<sub>PC</sub> = 5.3 Hz), 68.4 (d, <sup>2</sup>J<sub>PC</sub> = 5.3 Hz), 127.5, 128.4, 128.7, 129.5, 129.6, 130.4, 138.2, 142.4, 158.1, 173.8; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>):  $\delta$  5.06; (ESI): *m/z* 506.5 (M+H)<sup>+</sup>, 528.4 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>25</sub>H<sub>36</sub>N<sub>3</sub>O<sub>6</sub>P: C, 59.39; H, 7.18; N, 8.31. Found: C, 59.45; H, 7.27; N, 8.23.

**Phenyl hydrogen *N'*-(*S*)-2-(2-oxo-2-phenylethylideneamino)-3-phenyl-propanoyl-phosphorohydrazidate (**14**).**

To the stirred solution of acid **5** (299 mg, 1 mmol), hydrazine **13** (316 mg, 1.2 mmol) and HOBt (340 mg, 2.2 mmol) in THF (2.5 mL), DCC (230 mg, 1.1 mmol) was added at 0°C. The reaction mixture was allowed to

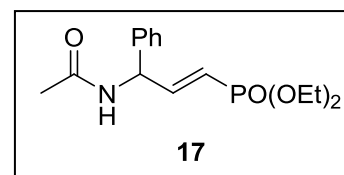


stir at 0°C for 2 h and further 12 h at rt. After completion of the reaction (TLC), the reaction mixture was filtered and concentrated in vacuum. The crude was redissolved in ethyl acetate (50 mL), washed with water (3x20 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to obtain the crude product which was purified by silica gel column chromatography using ethyl acetate: petroleum ether (1:1) as eluant to yield the pure product **14** (300 mg, 55%) as colourless syrup;  $[\alpha]_D^{20} = -3.2$  (*c* 1.0, MeOH); IR (CHCl<sub>3</sub>): 3291, 3186, 3020, 1688, 1598, 1215 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, acetone-d<sub>6</sub> + CDCl<sub>3</sub>):  $\delta$  3.07-3.27 (m, 2H), 4.53-4.69 (m, 1H), 5.07 (s, 2H), 7.32-7.98 (m, 15H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  42.4, 60.5, 71.0, 123.9, 125.6, 130.1, 131.8, 132.4, 132.9, 133.5, 134.5, 134.6, 134.8, 142.7, 147.8, 164.2, 175.2; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>):  $\delta$  3.20; (ESI): *m/z* 470.2 (M+H)<sup>+</sup>; Anal. Calcd for C<sub>23</sub>H<sub>24</sub>N<sub>3</sub>O<sub>6</sub>P: C, 58.85; H, 5.15; N, 8.95. Found: C, 58.76; H, 5.25; N, 8.74.

**(*E*)-Diethyl 3-acetamido-3-phenylprop-1-enylphosphonate (**17**).**

To the cooled solution of hydroxyphosphonate **16** (135 mg, 0.5 mmol) in acetonitrile (3 mL) at 0°C was added BF<sub>3</sub>·Et<sub>2</sub>O (0.4 mL, 48% w/v) drop wise. The reaction

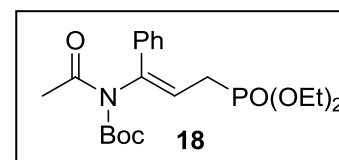
mixture was allowed to stir for 24 h at rt. After completion of the reaction (TLC), saturated aq.  $\text{NaHCO}_3$  (10 mL) was added and the product was extracted with DCM (3x10 mL). The combined organic layers was



dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure to furnish the crude product which was purified by silica gel column chromatography using ethyl acetate: petroleum ether (1:1 to 2:1) as eluant to furnish pure product **17** (220 mg, 70%) as colourless syrup; IR ( $\text{CHCl}_3$ ): 3439, 3019, 1673, 1495, 1395, 1215  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.29 (t,  $^3J_{\text{PH}} = 6.7$  Hz, 3H), 1.32 (t,  $^3J_{\text{PH}} = 6.9$  Hz, 3H), 2.04 (s, 3H), 3.99-4.14 (m, 4H), 5.74-5.94 (m, 2H), 6.54 (d,  $J = 8.1$  Hz, 1H), 6.77-6.98 (m, 1H), 7.24-7.38 (m, 5H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.3 (d,  $^3J_{\text{PC}} = 6.6$  Hz), 16.4 (d,  $^3J_{\text{PC}} = 6.2$  Hz), 23.2, 55.1 (d,  $^3J_{\text{PC}} = 22.3$  Hz), 61.9, 62.1, 117.5 (d,  $^1J_{\text{PC}} = 187.4$  Hz), 127.4, 128.2, 129.0, 138.9, 150.7 (d,  $^2J_{\text{PC}} = 5.9$  Hz), 169.3;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  17.67; (ESI):  $m/z$  312.5 ( $\text{M}+\text{H}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{15}\text{H}_{22}\text{NO}_4\text{P}$ : C, 57.87; H, 7.12; N, 4.50. Found: C, 57.92; H, 7.24; N, 4.61.

**(E)-Tert-butylacetyl(3-(diethoxyphosphoryl)-1-phenylprop-1-enyl)-carbamate (18).**

DMAP (12 mg, 0.1 mmol) was added to a stirred solution of compound **17** (155 mg, 0.5 mmol) in dry acetonitrile (3 mL) followed by  $\text{Boc}_2\text{O}$  (240 mg, 2.05 mmol). After stirring for 12 h at rt when all the starting material was

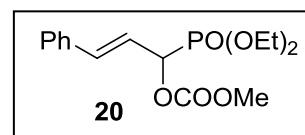


consumed (TLC), the brownish reaction mixture was evaporated under vacuum and the oily residue partitioned between diethyl ether (25 mL) and 1 M aq.  $\text{KHSO}_4$  (15 mL). The organic extract was thoroughly washed successively with 1 M aq. solution of  $\text{KHSO}_4$  (15 mL), saturated  $\text{NaHCO}_3$  (15 mL) and finally brine (15 mL) and dried over  $\text{Na}_2\text{SO}_4$ . Evaporation of the solvent furnished a light yellow oil, which was purified by silica gel column chromatography using ethyl acetate: petroleum ether (1:1) as eluant to furnish pure product **18** (170 mg, 83%) as colourless syrup; IR ( $\text{CHCl}_3$ ): 2981, 2934, 1738, 1713, 1446, 1394, 1370  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.26 (s, 9H), 1.34-1.35 (m, 6H), 2.53-2.69 (m, 2H), 2.60 (s, 3H), 4.11-4.19 (m, 4H), 6.07-6.19 (m, 1H), 7.31-7.33 (m, 5H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.4 (d,  $^3J_{\text{PC}} = 6.2$  Hz), 23.9, 24.3 (d,  $^1J_{\text{PC}} = 142.3$  Hz), 25.5, 59.9 (d,  $^2J_{\text{PC}} = 6.6$  Hz), 60.1

(d,  $^2J_{PC} = 6.6$  Hz), 81.3, 115.5 (d,  $^2J_{PC} = 8.4$  Hz), 123.0, 126.1, 126.4, 135.1 (d,  $^4J_{PC} = 2.2$  Hz), 137.0 (d,  $^3J_{PC} = 16.5$  Hz), 149.9, 169.6;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  26.52; (ESI):  $m/z$  434.4 ( $\text{M}+\text{Na}^+$ ); Anal. Calcd for  $\text{C}_{20}\text{H}_{30}\text{NO}_6\text{P}$ : C, 58.39; H, 7.35; N, 3.40. Found: C, 58.45; H, 7.42; N, 3.36.

**(E)-1-(Diethoxyphosphoryl)-3-phenylallyl methyl carbonate (20).**

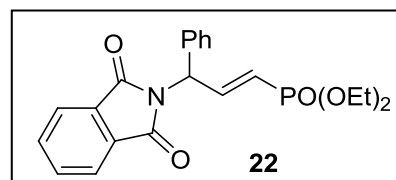
The hydroxy phosphonate **16** (387 mg, 1.4 mmol) was dissolved in a mixture of acetonitrile (10 mL) and pyridine (1 mL), and then DMAP (10 mg) was added. The resulting



solution was cooled to 0 °C, and methyl chloroformate (850  $\mu\text{L}$ , 9.0 mmol) was added drop wise. The solution was allowed to warm up to rt and then was stirred overnight. The solution was diluted with DCM (10 mL) and washed with water (2x10 mL) and saturated  $\text{CuSO}_4$  solution (4x10 mL). The solvent was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated in vacuum to give crude product which was purified by silica gel column chromatography using ethyl acetate: petroleum ether (3:7) as eluant to furnish pure product **20** (320 mg, 70%) as colourless syrup; IR ( $\text{CHCl}_3$ ): 3017, 1755, 1650, 1442, 1266, 1216, 1027  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.34 (t,  $^3J_{\text{PH}} = 7.1$  Hz, 6H), 3.84 (s, 3H), 4.13-4.28 (m, 4H), 5.59-5.70 (m, 1H), 6.20-6.34 (m, 1H), 6.75-6.85 (m, 1H), 7.28-7.44 (m, 5H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.4 (d,  $^3J_{PC} = 5.8$  Hz), 16.5 (d,  $^3J_{PC} = 5.5$  Hz), 55.4, 63.4 (d,  $^2J_{PC} = 6.6$  Hz), 63.4 (d,  $^2J_{PC} = 7.3$  Hz), 73.4 (d,  $^1J_{PC} = 170.9$  Hz), 119.6 (d,  $^2J_{PC} = 4.4$  Hz), 126.9, 128.5, 128.7, 135.4 (d,  $^3J_{PC} = 12.8$  Hz), 135.6 (d,  $^4J_{PC} = 2.5$  Hz), 154.8 (d,  $^3J_{PC} = 9.8$  Hz);  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.64; (ESI):  $m/z$  351.2 ( $\text{M}+\text{Na}^+$ ); Anal. Calcd for  $\text{C}_{15}\text{H}_{21}\text{O}_6\text{P}$ : C, 54.88; H, 6.45. Found: C, 54.75; H, 6.57.

**(E)-Diethyl 3-(1,3-dioxoisindolin-2-yl)-3-phenylprop-1-enylphosphonate (22).**

To a flask purged with nitrogen,  $\text{Pd}(\text{OAc})_2$  (8 mg, 0.03 mmol),  $\text{PPh}_3$  (19 mg, 0.07 mmol) and compound **20** (230 mg, 0.7 mmol) dissolved in DMF (5 mL) were slowly added. Then the reaction mixture was stirred at

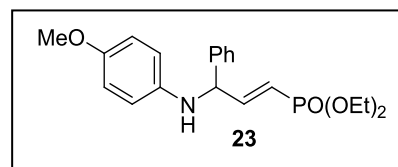


80 °C for 3 h. After completion of the reaction (TLC), diethyl ether (30 mL) was added. The mixture was washed with water (3x20 mL), the organic layer was dried

over  $\text{Na}_2\text{SO}_4$  and concentrated in vacuum. The residue was purified by column chromatography over silica gel by using ethyl acetate: petroleum ether (2:3) as eluant to provide pure product **22** (93 mg, 33%) as colourless syrup; IR ( $\text{CHCl}_3$ ): 2986, 2932, 1771, 1714, 1634, 1613, 1469, 1355, 1245  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.31 (t,  $^3J_{\text{PH}} = 7.1$  Hz, 3H), 1.37 (t,  $^3J_{\text{PH}} = 7.1$  Hz, 3H), 4.05-4.21 (m, 4H), 5.71-5.90 (m, 1H), 6.04-6.10 (m, 1H), 7.22-7.86 (m, 10H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.3, 16.4, 56.1 (d,  $^3J_{\text{PC}} = 24.5$  Hz), 62.1 (d,  $^3J_{\text{PC}} = 5.5$  Hz), 62.2 (d,  $^3J_{\text{PC}} = 5.5$  Hz), 120.6 (d,  $^1J_{\text{PC}} = 186.9$  Hz), 123.6, 128.4, 128.5, 128.6, 128.7, 128.9, 131.7, 132.0, 132.2, 134.3, 136.8, 147.6 (d,  $^2J_{\text{PC}} = 6.6$  Hz), 167.4;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.94; (ESI):  $m/z$  422.2 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{21}\text{H}_{22}\text{NO}_5\text{P}$ : C, 63.15; H, 5.55; N, 3.51. Found: C, 63.27; H, 5.63; N, 3.43.

**(E)-Diethyl 3-(4-methoxyphenylamino)-3-phenylprop-1-enylphosphonate (23).**

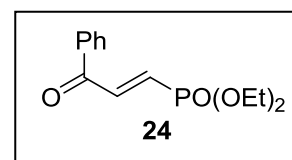
A solution of compound **20** (328 mg, 1mmol) in THF (3 mL) was stirred at rt under nitrogen, and then  $\text{Pd}(\text{OAc})_2$  (11 mg, 5 mol%) and  $\text{PPh}_3$  (27 mg, 10 mol%) were added to it. After stirring for 10 min,



*p*-anisidine (246 mg, 2 mmol) was added and the reaction was further stirred at rt. After completion of the reaction (TLC), reaction mixture was evaporated to give the crude compound, which was purified by flash chromatography over silica gel using ethyl acetate: petroleum ether (2:3) as eluant to obtain pure compound **23** (355 mg, 95%) as yellow syrup; IR ( $\text{CHCl}_3$ ): 3419, 2995, 1633, 1512, 1454, 1242, 1216  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.26 (t,  $^3J_{\text{PH}} = 7.1$  Hz, 3H), 1.36 (t,  $^3J_{\text{PH}} = 7.1$  Hz, 3H), 3.73 (s, 3H), 3.96-4.11 (m, 4H), 4.94-4.99 (m, 1H), 5.97 (ddd,  $J = 19.9, 1.6, 17.1$  Hz, 1H), 6.52-6.77 (m, 4H), 6.93 (ddd,  $J = 4.7, 17.1, 21.6$  Hz, 1H), 7.29-7.68 (m, 5H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.3 (d,  $^3J_{\text{PC}} = 6.2$  Hz), 55.7, 61.6 (d,  $^3J_{\text{PC}} = 26.4$  Hz), 61.8, 61.9, 114.8, 114.9, 117.4 (d,  $^1J_{\text{PC}} = 186.6$  Hz), 127.4, 128.1, 129.0, 139.8, 140.8, 152.2 (d,  $^2J_{\text{PC}} = 5.8$  Hz), 152.5;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  18.40; (ESI):  $m/z$  398.1 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{20}\text{H}_{26}\text{NO}_4\text{P}$ : C, 63.99; H, 6.98; N, 3.73. Found: C, 63.90; H, 6.86; N, 3.65.

**(E)-Diethyl 3-oxo-3-phenylprop-1-enylphosphonate (24).**

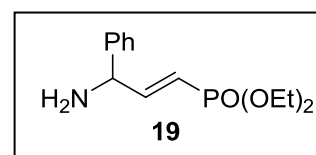
To a solution of **23** (240 mg, 0.64 mmol) in acetonitrile (4.0 mL), aqueous cerium ammonium nitrate solution (1800 mg, 3.2 mmol, 4.0 mL) was added at 0 °C. After being stirred at 0



°C for 1 h, the reaction mixture was quenched by addition of sat. NaHCO<sub>3</sub> solution. The aqueous layer was extracted with diethyl ether (3x25 mL) and the combined organic layers were successively washed with sat. NaHCO<sub>3</sub> solution (20 mL), brine (20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solvent was evaporated in vacuum to afford the crude material, which was purified by flash chromatography over silica gel using ethyl acetate: petroleum ether (2:3) as eluant to furnish the pure compound **24** (16 mg, 10%) as a colourless syrup; IR (CHCl<sub>3</sub>): 3019, 1672, 1597, 1448, 1259, 1216 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 1.37 (t, <sup>3</sup>J<sub>PH</sub> = 7.1 Hz, 6H), 4.10-4.25 (m, 4H), 6.95 (dd, *J* = 16.9, 19.3 Hz, 1H), 7.45-7.64 (m, 3H), 7.83 (dd, *J* = 16.9, 21.1 Hz, 1H), 7.99-8.04 (m, 2H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ 16.4, 62.5, 128.9, 129.0, 130.8 (d, <sup>1</sup>J<sub>PC</sub> = 184.1 Hz), 133.9, 136.3, 140.2 (d, <sup>2</sup>J<sub>PC</sub> = 5.9 Hz), 188.5 (d, <sup>3</sup>J<sub>PC</sub> = 22.0 Hz); <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 15.78; (ESI): *m/z* 291.2 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>13</sub>H<sub>17</sub>O<sub>4</sub>P: C, 58.21; H, 6.39. Found: C, 58.15; H, 6.29.

**(E)-Diethyl 3-amino-3-phenylprop-1-enylphosphonate (19) or its HCl salt (25).**

To a solution of **23** (150 mg, 0.4 mmol) in MeCN/H<sub>2</sub>O (10 mL, 1:1) were added TCCA (46 mg, 0.2 mmol) and 1 M aqueous H<sub>2</sub>SO<sub>4</sub> (0.4 mL). The mixture was stirred for 12 h at rt and then washed with CH<sub>2</sub>Cl<sub>2</sub> (3x20 mL). The resulting

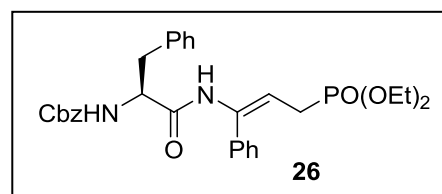


aqueous phase was subsequently brought to pH 10.5 through the addition of 5 M aqueous KOH and extracted with EtOAc (4x30 mL). The combined organic layers were brought to pH 1 by the addition of EtOAc/HCl, dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated to afford the HCl salt **25**. In an alternative work up procedure, the combined organic layers were evaporated in vacuum to furnish crude material, which was purified by flash chromatography over silica gel using ethyl acetate as eluant to afford pure free amine **19** (63 mg, 59%) as yellow syrup; IR (CHCl<sub>3</sub>): 3420, 2985, 2641, 1636, 1605, 1532, 1456, 1343, 1217 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 1.31 (t, <sup>3</sup>J<sub>PH</sub> = 7.1 Hz, 3H), 1.34 (t, <sup>3</sup>J<sub>PH</sub> = 7.1 Hz, 3H), 4.01-4.10 (m, 4H), 4.61 (bs, 2H), 5.13 (m, 1H), 6.08 (m, 1H), 6.96 (m, 1H), 7.45 (m, 5H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ 15.6, (d, <sup>3</sup>J<sub>PC</sub> = 6.2 Hz), 56.0 (d, <sup>3</sup>J<sub>PC</sub> = 24.2 Hz), 62.4 (d, <sup>2</sup>J<sub>PC</sub> = 5.5 Hz), 120.5 (d, <sup>1</sup>J<sub>PC</sub> = 186.3 Hz),

127.3, 129.0, 129.3, 133.7, 145.9 (d,  $^2J_{PC} = 5.9$  Hz);  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.1; (ESI):  $m/z$  270.7 ( $\text{M}+\text{H}$ ) $^+$ , 292.7 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{13}\text{H}_{20}\text{NO}_3\text{P}$ : C, 57.98; H, 7.49; N, 5.20. Found: C, 57.83; H, 7.38; N, 5.15.

**(*S,E*)-Benzyl 1-(3-(diethoxyphosphoryl)-1-phenylprop-1-enylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (**26**).**

HCl salt **25** (152 mg, 0.5 mmol), HOBt (77 mg, 0.5 mmol), acid **5** (0.5 mmol) and  $\text{Et}_3\text{N}$  (50 mg, 0.5 mmol) were dissolved in dry THF (2 mL) and the solution was stirred in an ice water bath and



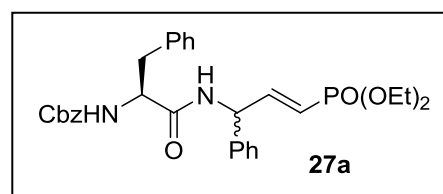
then DCC (123 mg, 0.6 mmol) was added to it. Stirring was continued for 1 h at  $0^\circ\text{C}$  and an additional 1 h at rt. The solid which separated was removed by filtration and the solvent evaporated in vacuo. A mixture of ethyl acetate (5 mL) and saturated solution of  $\text{NaHCO}_3$  in water (2.5 mL) were added to the residue and the organic phase was washed successively with a solution of aq. citric acid (10 %, 5 mL), saturated  $\text{NaHCO}_3$  (5 mL) and finally with water (5 mL). The organic solution was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and evaporated to dryness in vacuum. The crude dipeptide derivative was purified by chromatography on a silica gel column with ethyl acetate: petroleum ether (4:1) as eluant to furnish **26** (140 mg, 52%) as colourless syrup;  $[\alpha]_D^{20} = -11.0$  ( $c$  1.00,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ ): 3423, 3300, 3018, 1715, 1685, 1496, 1216, 1053, 759  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.26 (t,  $^3J_{\text{PH}} = 7.1$  Hz, 6H), 2.35-2.72 (m, 2H), 3.00-3.15 (m, 2H), 3.94-4.10 (m, 4H), 4.49-4.64 (m, 1H), 5.08 (s, 2H), 5.53-5.63 (m, 1H), 5.72 (bs, 1H), 7.05 (bs, 1H), 7.13-7.31 (m, 15H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.4 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 16.5 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 25.7 (d,  $^1J_{\text{PC}} = 138.7$  Hz), 38.2, 56.6, 62.4 (d,  $^2J_{\text{PC}} = 7.0$  Hz), 62.6 (d,  $^2J_{\text{PC}} = 7.0$  Hz), 66.9, 111.2 (d,  $^2J_{\text{PC}} = 6.6$  Hz), 125.9, 126.0, 127.1, 127.3, 127.4, 128.0, 128.1, 128.3, 128.3, 128.5, 128.8, 128.9, 129.0, 129.4, 129.6, 136.4, 136.8 (d,  $^4J_{\text{PC}} = 4.0$  Hz), 136.3, 139.3 (d,  $^3J_{\text{PC}} = 13.5$  Hz), 156.1, 170.2;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.34; (ESI):  $m/z$  573.7 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{30}\text{H}_{35}\text{N}_2\text{O}_6\text{P}$ : C, 65.44; H, 6.41; N, 5.09. Found: C, 65.54; H, 6.35; N, 5.18.

**General procedure for peptide coupling reaction:**

Compound **19** (135 mg, 0.5 mmol), HOBt (77 mg, 0.5 mmol) and protected amino acid (0.5 mmol) were dissolved in dry THF (2 mL) and the resulting solution was stirred in an ice-cooled water bath. DCC (123 mg, 0.6 mmol) was added to it. Stirring was continued for 1 h at 0°C and an additional 1 h at rt. The solid which separated was removed by filtration and the solvent evaporated in vacuum. The crude dipeptide derivative was purified by chromatography on a silica gel column with ethyl acetate: petroleum ether (3:2 to 4:1) as eluant to furnish the corresponding peptide **27a-j**.

**Benzyl (2S)-1-((E)-3-(diethoxyphosphoryl)-1-phenylallylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (27a)** (only peaks corresponding to the major isomer are given).

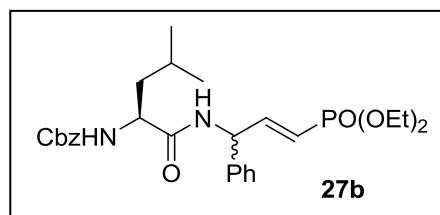
Yield: 53%; colourless syrup; IR (CHCl<sub>3</sub>): 3424, 3019, 1713, 1676, 1497, 1395, 1215 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 1.17-1.33 (m, 6H), 3.04 (dd, *J* = 6.4, 5.4 Hz, 2H), 3.90-4.09 (m, 4H),



4.52-4.54 (m, 1H), 4.94-5.00 (m, 2H), 5.53-5.95 (m, 2H), 5.71-5.72 (bs, 1H), 6.65-7.00 (m, 1H), 7.07-7.42 (m, 15H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ 16.3, 16.4, 38.9, 55.0 (d, <sup>3</sup>*J*<sub>PC</sub> = 21.6 Hz), 56.2, 61.9 (d, <sup>2</sup>*J*<sub>PC</sub> = 5.8 Hz), 62.1 (d, <sup>2</sup>*J*<sub>PC</sub> = 5.8 Hz), 66.9, 117.6 (d, <sup>1</sup>*J*<sub>PC</sub> = 187.4 Hz), 126.9, 127.1, 127.3, 127.4, 128.0, 128.1, 128.2, 128.5, 128.7, 128.9, 129.4, 136.2, 136.3, 138.7, 150.4 (d, <sup>2</sup>*J*<sub>PC</sub> = 4.7 Hz), 155.9, 170.4; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 17.40; (ESI): *m/z* 573.4 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>30</sub>H<sub>35</sub>N<sub>2</sub>O<sub>6</sub>P: C, 65.44; H, 6.41; N, 5.09. Found: C, 65.55; H, 6.33; N, 5.23.

**Benzyl (2S)-1-((E)-3-(diethoxyphosphoryl)-1-phenylallylamino)-4-methyl-1-oxopentan-2-ylcarbamate (27b)** (only peaks corresponding to the major isomer are given).

Yield: 76%; colourless syrup; IR (CHCl<sub>3</sub>): 3423, 3264, 3017, 2960, 2933, 1718, 1685, 1499, 1509, 1449, 1217, 1028 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 0.85-0.88 (m, 6H), 1.15-1.34 (m, 6H),



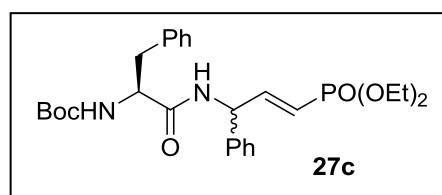
1.52-1.64 (m, 3H), 3.88-4.12 (m, 4H), 4.33-4.37 (m, 1H), 4.98-5.06 (m, 2H), 5.73-5.79 (m, 1H), 5.85-6.05 (m, 1H), 6.72-6.98 (m, 1H), 7.24-7.30 (m, 10H); <sup>13</sup>C NMR



(50 MHz, CDCl<sub>3</sub>):  $\delta$  16.2 (d,  $^3J_{\text{PC}} = 6.6$  Hz), 16.3 (d,  $^3J_{\text{PC}} = 6.6$  Hz), 22.9, 23.0, 24.7, 41.8, 53.7, 54.9 (d,  $^3J_{\text{PC}} = 22.7$  Hz), 62.1 (d,  $^2J_{\text{PC}} = 9.9$  Hz), 62.0 (d,  $^2J_{\text{PC}} = 9.9$  Hz), 66.9, 117.2 (d,  $^1J_{\text{PC}} = 187.4$  Hz), 127.3, 127.9, 128.1, 128.5, 128.7, 128.9, 136.2, 138.8, 150.8 (d,  $^2J_{\text{PC}} = 5.5$  Hz), 156.2, 172.0;  $^{31}\text{P}$  NMR (161 MHz, CDCl<sub>3</sub>):  $\delta$  17.64; (ESI):  $m/z$  517.4 (M+H)<sup>+</sup>; Anal. Calcd for C<sub>27</sub>H<sub>35</sub>N<sub>2</sub>O<sub>6</sub>P: C, 62.78; H, 7.22; N, 5.42. Found: C, 62.68; H, 7.32; N, 5.35.

**Tert-butyl (2S)-1-((E)-3-(diethoxyphosphoryl)-1-phenylallylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (27c)** (only peaks corresponding to the major isomer are given).

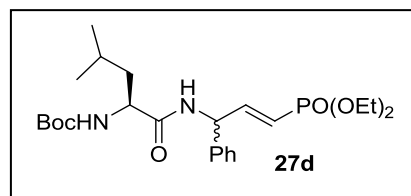
Yield: 77%; colourless syrup; IR (CHCl<sub>3</sub>): 3427, 3018, 1680, 1495, 1393, 1368, 1216, 1165, 1052, 1029 cm<sup>-1</sup>;  $^1\text{H}$  NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  1.22-1.34 (m, 6H), 1.35 (s, 9H), 3.04-3.08 (m, 2H),



3.96-4.11 (m, 4H), 4.40-4.44 (m, 1H), 5.22-5.42 (m, 1H), 5.62-5.92 (m, 1H), 6.67-6.93 (m, 1H), 7.10-7.29 (m, 10H);  $^{13}\text{C}$  NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  16.3, 16.4, 28.2, 38.3, 54.9 (d,  $^3J_{\text{PC}} = 22.7$  Hz), 56.2, 62.0, 62.1, 80.1, 116.9 (d,  $^1J_{\text{PC}} = 187.7$  Hz), 125.2, 126.8, 126.9, 127.3, 128.1, 128.6, 128.7, 128.9, 128.9, 129.3, 136.4, 138.5, 150.6 (d,  $^2J_{\text{PC}} = 5.9$  Hz), 155.5, 170.8;  $^{31}\text{P}$  NMR (161 MHz, CDCl<sub>3</sub>):  $\delta$  17.70; (ESI):  $m/z$  539.4 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>27</sub>H<sub>37</sub>N<sub>2</sub>O<sub>6</sub>P: C, 62.78; H, 7.22; N, 5.42. Found: C, 62.88; H, 7.36; N, 5.35.

**Tert-butyl (2S)-1-((E)-3-(diethoxyphosphoryl)-1-phenylallylamino)-4-methyl-1-oxopentan-2-ylcarbamate (27d)** (only peaks corresponding to the major isomer are given).

Yield: 62%; colourless syrup; IR (CHCl<sub>3</sub>): 3432, 3019, 1688, 1496, 1392, 1369, 1216, 1028 cm<sup>-1</sup>;  $^1\text{H}$  NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  0.88-0.94 (m, 6H), 1.23-1.34 (m, 6H), 1.38 (s, 9H), 1.48-1.69 (m, 3H),

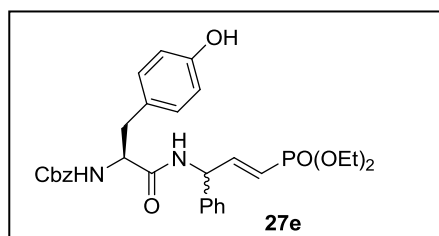


3.97-4.15 (m, 4H), 5.06-5.31 (m, 1H), 5.72-5.92 (m, 2H), 6.74-6.99 (m, 1H), 7.22-7.39 (m, 5H);  $^{13}\text{C}$  NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  16.2, 16.3, 21.9, 22.9, 24.7, 28.2, 40.7, 53.2, 54.9 (d,  $^3J_{\text{PC}} = 22.3$  Hz), 62.0, 62.1, 80.1, 117.1 (d,  $^1J_{\text{PC}} = 187.0$  Hz), 127.3, 128.1, 128.9, 138.6, 150.8 (d,  $^2J_{\text{PC}} = 5.9$  Hz), 155.9, 172.0;  $^{31}\text{P}$  NMR (161 MHz,

$\text{CDCl}_3$ ):  $\delta$  17.80; (ESI):  $m/z$  505.4 ( $\text{M}+\text{Na}$ )<sup>+</sup>; Anal. Calcd for  $\text{C}_{24}\text{H}_{39}\text{N}_2\text{O}_6\text{P}$ : C, 59.74; H, 8.15; N, 5.81. Found: C, 59.81; H, 8.02; N, 5.93.

**Benzyl (2S)-1-((E)-3-(diethoxyphosphoryl)-1-phenylallylamino)-3-(4-hydroxyphenyl)-1-oxopropan-2-ylcarbamate (27e)** (only peaks corresponding to the major isomer are given).

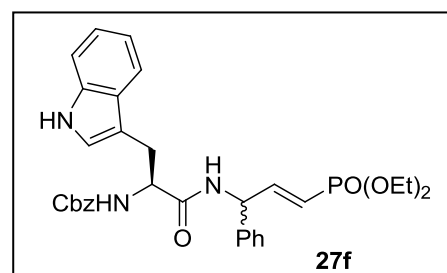
Yield: 60%; colourless syrup; IR ( $\text{CHCl}_3$ ): 3422, 3287, 3018, 1712, 1672, 1515, 1454, 1216, 1027  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.20 (t,  $^3J_{\text{PH}} = 6.9$  Hz, 6H), 1.26 (t,  $^3J_{\text{PH}} = 6.9$  Hz, 3H), 2.83-3.05 (m, 2H), 3.91-4.12 (m, 4H), 4.39-4.55 (m,



1H), 4.93 (s, 2H), 5.59-5.88 (m, 2H), 6.60-7.49 (m, 15H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.2, 16.3, 37.9, 55.2 (d,  $^3J_{\text{PC}} = 22.7$  Hz), 56.5, 62.4, 67.1, 117.5 (d,  $^1J_{\text{PC}} = 187.4$  Hz), 126.0, 126.7, 127.3, 127.9, 128.2, 128.5, 128.7, 128.9, 129.0, 130.3, 130.4, 134.1, 136.0, 138.2, 150.7 (d,  $^2J_{\text{PC}} = 5.1$  Hz), 155.9, 171.0;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  17.71; (ESI):  $m/z$  589.0 ( $\text{M}+\text{Na}$ )<sup>+</sup>; Anal. Calcd for  $\text{C}_{30}\text{H}_{35}\text{N}_2\text{O}_6\text{P}$ : C, 63.60; H, 6.23; N, 4.94. Found: C, 63.72; H, 6.32; N, 5.10.

**Benzyl (2S)-1-((E)-3-(diethoxyphosphoryl)-1-phenylallylamino)-3-(1H-indol-3-yl)-1-oxopropan-2-ylcarbamate (27f)** (only peaks corresponding to the major isomer are given).

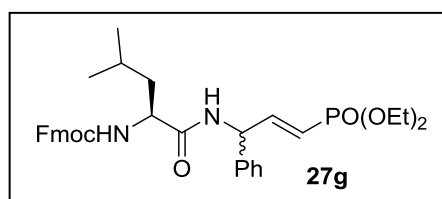
Yield: 60%; colourless syrup; IR ( $\text{CHCl}_3$ ): 3419, 3297, 3019, 2933, 1712, 1675, 1500, 1216, 1051  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.21-1.36 (m, 6H), 3.04-3.39 (m, 2H), 3.91-4.14 (m, 4H), 4.52-4.69 (m, 1H), 5.06 (s, 2H), 5.23-5.70 (m, 2H), 6.27-6.89 (m, 1H), 6.84-7.72 (m, 15H);  $^{13}\text{C}$  NMR



(50 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.3 (d,  $^3J_{\text{PC}} = 6.4$  Hz), 16.4 (d,  $^3J_{\text{PC}} = 6.4$  Hz), 28.2, 54.9 (d,  $^3J_{\text{PC}} = 23.6$  Hz), 55.6, 62.1, 62.2 (d,  $^2J_{\text{PC}} = 5.5$  Hz), 66.9, 109.9, 111.9, 117.6 (d,  $^1J_{\text{PC}} = 187.1$ , Hz), 118.8, 119.8, 122.1, 122.2, 124.1, 124.3, 127.1, 127.3, 128.1, 128.2, 128.2, 128.6, 128.9, 129.0, 136.2, 136.6, 137.8, 138.1, 150.1 (d,  $^2J_{\text{PC}} = 4.5$  Hz), 155.8, 170.7;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  17.90; (ESI):  $m/z$  612.3 ( $\text{M}+\text{Na}$ )<sup>+</sup>; Anal. Calcd for  $\text{C}_{32}\text{H}_{36}\text{N}_3\text{O}_6\text{P}$ : C, 65.18; H, 6.15; N, 7.13. Found: C, 65.24; H, 6.21; N, 7.22.

**(9H-Fluoren-9-yl)methyl (2S)-1-((E)-3-(diethoxyphosphoryl)-1-phenylallylamino)-4-methyl-1-oxopentan-2-ylcarbamate (27g)** (only peaks corresponding to the major isomer are given).

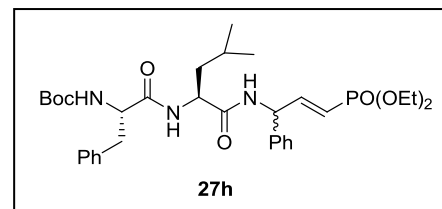
Yield: 85%; colourless syrup; IR (CHCl<sub>3</sub>): 3274, 3065, 2958, 1716, 1670, 1541, 1450, 1239 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 0.89-0.92 (m, 6H), 1.17-1.29 (m, 6H), 1.60-1.69 (m, 3H), 3.90-



4.20 (m, 5H), 4.32-4.39 (m, 3H), 5.48-6.28 (m, 2H), 6.74-6.98 (m, 1H), 7.22-7.84 (m, 13H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ 16.2 (d, <sup>3</sup>J<sub>PC</sub> = 6.2 Hz), 16.3 (d, <sup>3</sup>J<sub>PC</sub> = 6.2 Hz), 21.8, 23.1, 24.8, 41.3, 47.1, 53.9, 54.9, 62.3, 62.4, 67.1, 117.7 (d, <sup>1</sup>J<sub>PC</sub> = 186.9 Hz), 119.9, 125.0, 125.1, 125.8, 126.8, 127.1, 127.3, 127.7, 128.2, 129.0, 129.0, 138.3, 141.3, 141.4, 143.7, 143.9, 151.0, 156.5, 171.8; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 17.95; (ESI): *m/z* 627.8 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>34</sub>H<sub>41</sub>N<sub>2</sub>O<sub>6</sub>P: C, 67.53; H, 6.83; N, 4.63. Found: C, 67.64; H, 6.91; N, 4.70.

***Tert*-butyl (S)-1-((S)-1-((E)-3-(diethoxyphosphoryl)-1-((phenylallylamino)-4-methyl-1-oxopentan-2-ylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (27h)** (only peaks corresponding to the major isomer are given).

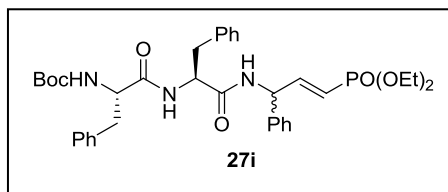
Yield: 54%; colourless syrup; IR (CHCl<sub>3</sub>): 3633, 3424, 3019, 1698, 1497, 1392, 1369, 1216, 1028 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 0.83-0.90 (m, 6H), 1.22-1.37 (m, 15H), 1.49-1.69 (m, 3H), 2.97-



3.06 (m, 2H), 4.01-4.11 (m, 4H), 4.25-4.35 (m, 1H), 4.44-4.55 (m, 1H), 5.03-5.07 (m, 1H), 5.74-5.98 (m, 2H), 6.70-7.00 (m, 1H), 7.13-7.34 (m, 10H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ 16.3, 16.4, 21.8, 22.9, 24.7, 28.1, 37.8, 40.7, 52.0, 52.1, 55.2 (d, <sup>3</sup>J<sub>PC</sub> = 27.8 Hz), 61.9, 62.1, 80.6, 117.4 (d, <sup>2</sup>J<sub>PC</sub> = 186.6 Hz), 127.0, 127.1, 127.4, 127.6, 128.1, 128.7, 128.8, 128.9, 129.2, 129.2, 136.2, 138.6, 150.6 (d, <sup>1</sup>J<sub>PC</sub> = 5.8 Hz), 155.7, 170.9, 171.6; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 17.72; (ESI): *m/z* 652.8 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>33</sub>H<sub>48</sub>N<sub>3</sub>O<sub>7</sub>P: C, 62.94; H, 7.68; N, 6.67. Found: C, 62.83; H, 7.77; N, 6.78.

***Tert*-butyl (S)-1-((S)-1-((E)-3-(diethoxyphosphoryl)-1-phenylallylamino)-1-oxo-3-phenylpropan-2-ylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (27i)** (only peaks corresponding to the major isomer are given).

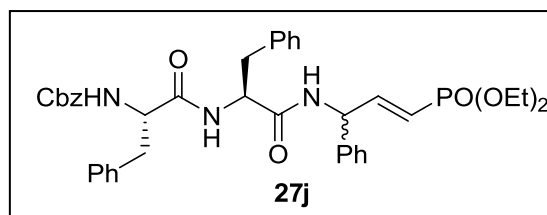
Yield: 50%; colourless syrup; IR (CHCl<sub>3</sub>): 3421, 3286, 3019, 2932, 1685, 1497, 1369, 1216, 1029 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  1.15-1.36 (m, 15H), 2.82-3.31 (m, 4H), 3.99-4.12 (m, 4H), 4.67-



4.97 (m, 2H), 5.65-5.83 (m, 2H), 6.36-6.79 (m, 1H), 6.90-7.30 (m, 15H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  16.3 (d, <sup>3</sup>J<sub>PC</sub> = 6.2 Hz), 16.4, (d, <sup>3</sup>J<sub>PC</sub> = 6.2 Hz), 28.2, 37.5, 37.7, 53.9, 55.0 (d, <sup>3</sup>J<sub>PC</sub> = 22.7 Hz), 56.1, 61.9, 62.0 (d, <sup>2</sup>J<sub>PC</sub> = 5.5 Hz), 80.6, 117.4 (d, <sup>1</sup>J<sub>PC</sub> = 185.5 Hz), 127.1, 127.2, 127.3, 127.4, 127.7, 128.1, 128.7, 128.8, 128.9, 129.2, 129.3, 135.9, 136.2, 138.2, 150.3, 155.6, 169.8, 171.1; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>):  $\delta$  17.72; (ESI): *m/z* 686.7 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>36</sub>H<sub>46</sub>N<sub>3</sub>O<sub>7</sub>P: C, 65.14; H, 6.99; N, 6.33. Found: C, 65.27; H, 6.86; N, 6.42.

**Benzyl (S)-1-((S)-1-((E)-3-(diethoxyphosphoryl)-1-phenylallylamino)-1-oxo-3-phenylpropan-2-ylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (27j)** (only peaks corresponding to the major isomer are given).

Yield: 43%; colourless syrup; IR (CHCl<sub>3</sub>): 3414, 3295, 3066, 3019, 1713, 1659, 1651, 1497, 1455, 1216 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  1.18-1.34 (m, 6H), 2.87-



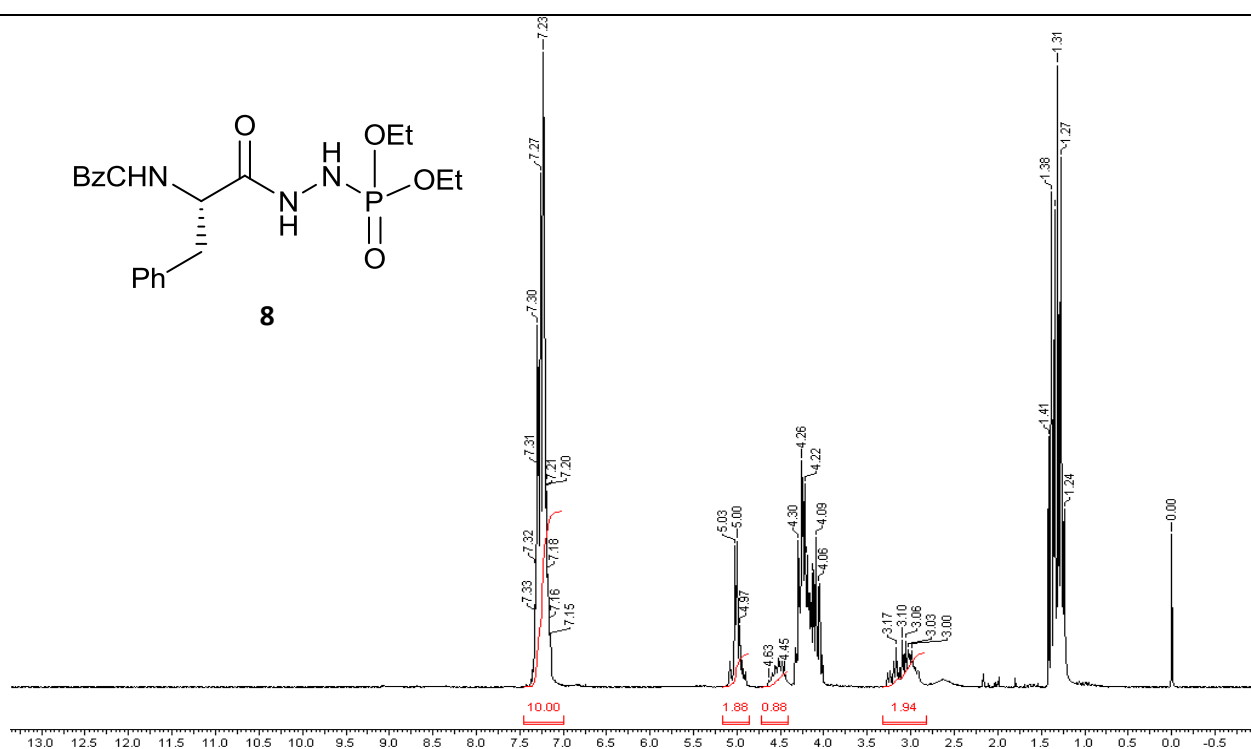
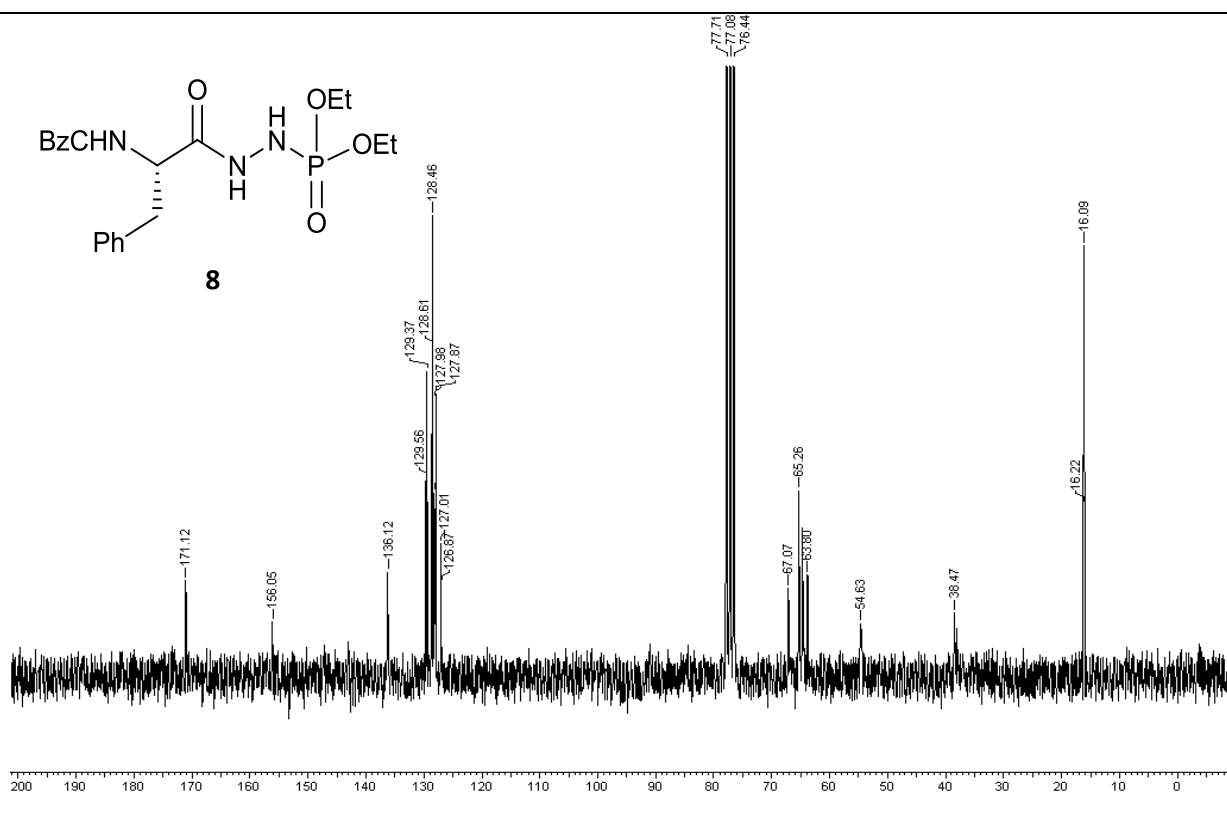
3.37 (m, 4H), 3.94-4.08 (m, 4H), 4.29-4.40 (m, 1H), 4.64-5.04 (m, 3H), 5.41-5.88 (m, 2H), 6.57-6.80 (m, 1H), 6.84-7.42 (m, 20H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  16.3, 16.4, 37.8, 38.0, 54.0, 54.9 (d, <sup>3</sup>J<sub>PC</sub> = 22.7 Hz), 56.5, 61.9, 62.1, 67.2, 117.3 (d, <sup>1</sup>J<sub>PC</sub> = 186.3 Hz), 126.9, 127.1, 127.2, 127.4, 127.5, 128.1, 128.2, 128.3, 128.6, 128.7, 128.8, 128.9, 129.2, 129.2, 129.3, 135.8, 135.9, 136.0, 138.5, 150.6, 156.2, 169.7, 170.8; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>):  $\delta$  17.84; (ESI): *m/z* 720.5 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>39</sub>H<sub>44</sub>N<sub>3</sub>O<sub>7</sub>P: C, 67.13; H, 6.36; N, 6.02. Found: C, 67.27; H, 6.43; N, 6.12.

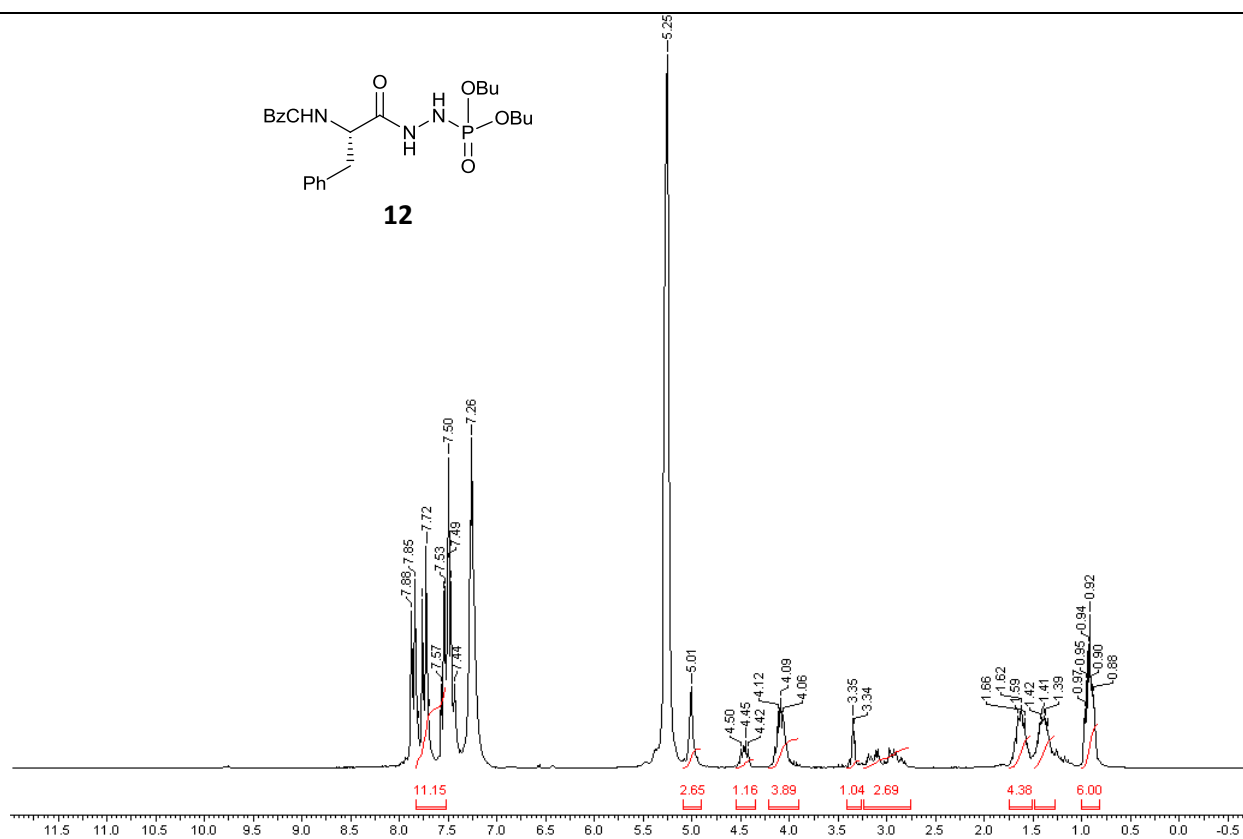
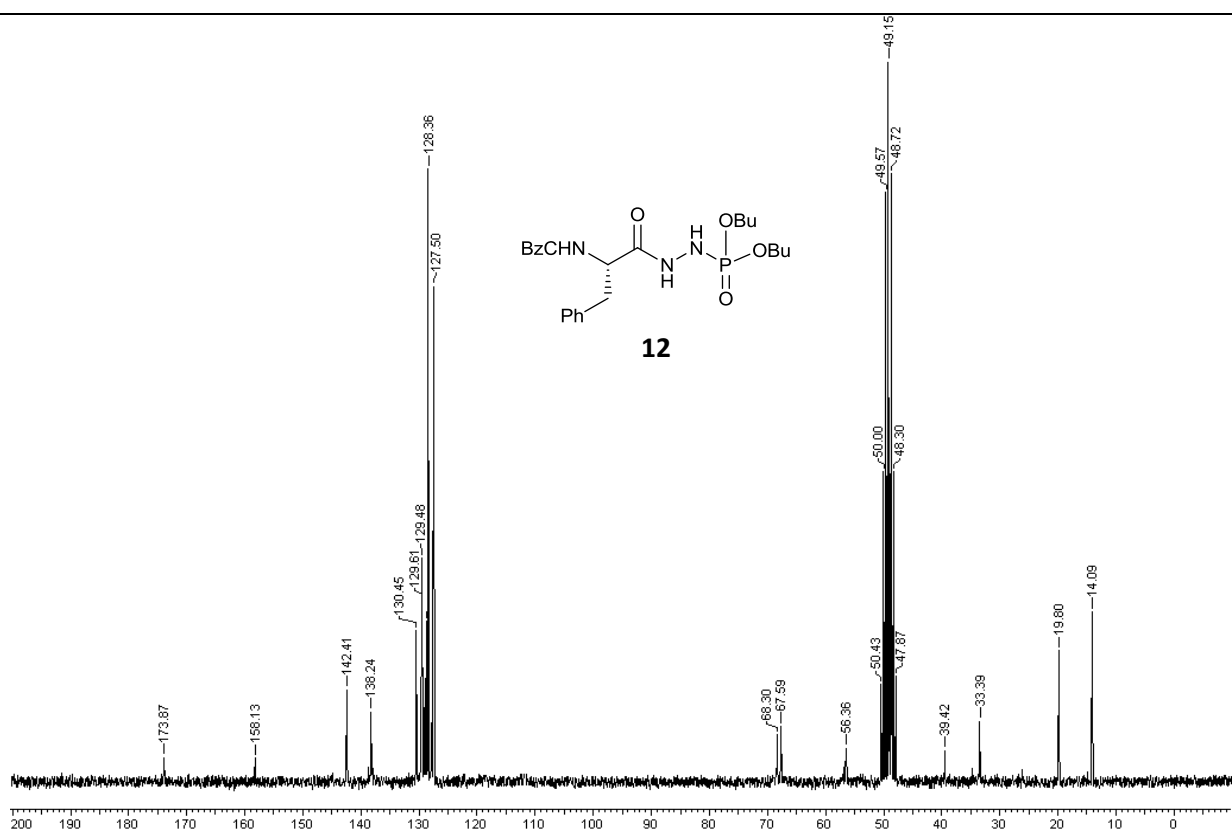
### Bioassay of Synthesized Peptidyl-vinylaminophosphonates 27a-j

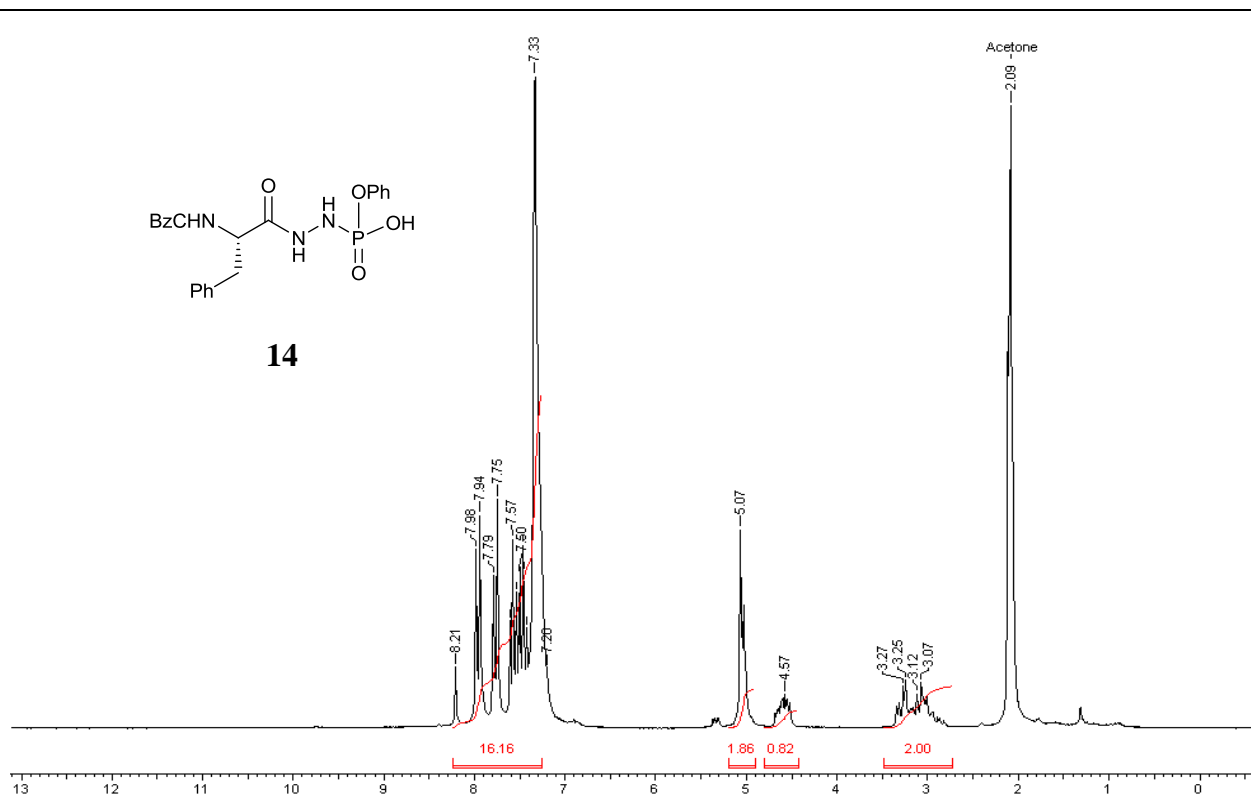
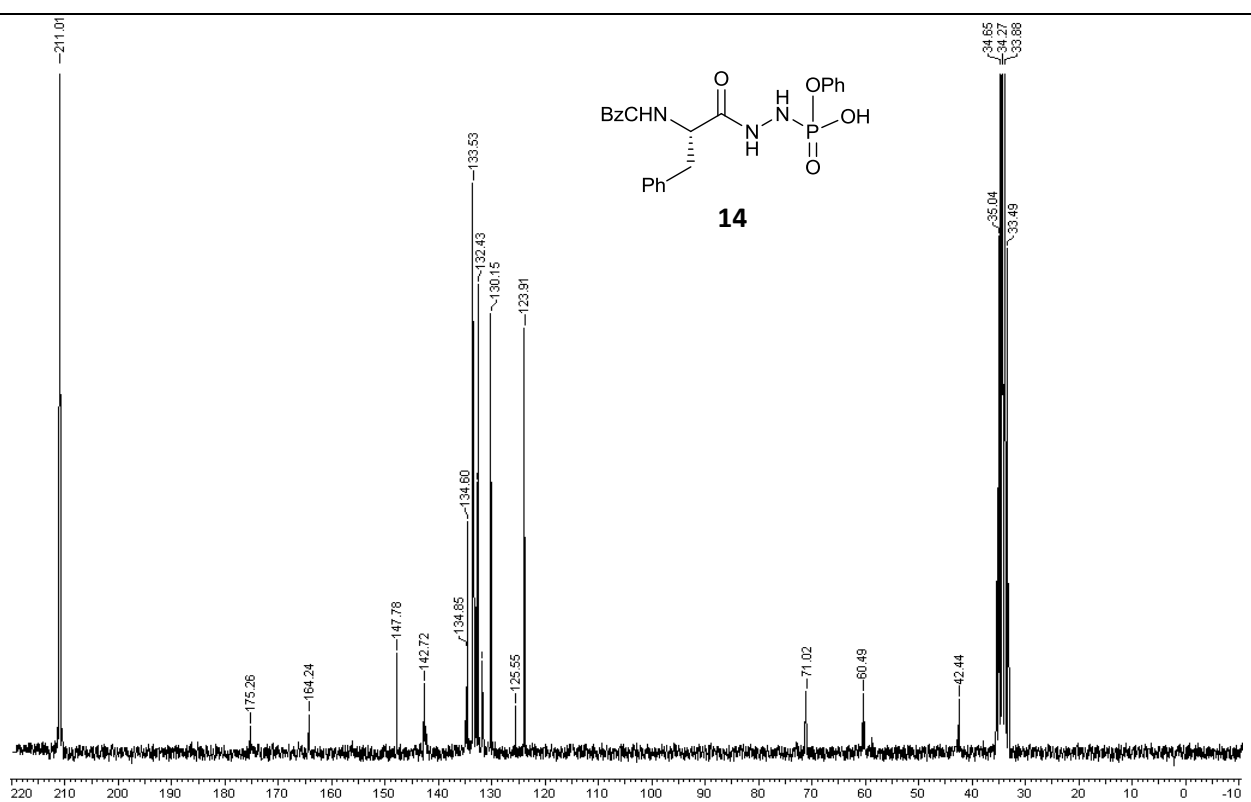
The incubation method was used to measure the inhibition of papain.<sup>9p</sup> The inhibition incubation buffer for papain was 100 mM Tris-HCl buffer at pH 6.5 containing 0.5 mM DTT (dithiothreitol) and 0.4 mM EDTA. The assay uses the substrate BAPNA (0.75 mM) in the same buffer. The approximate final concentration

of papain in enzymatic reaction was 0.04 mg/mL. The release of *p*-nitroanilide was monitored spectrophotometrically at 410 nm in the absence and presence of inhibitor. All assays were run in duplicate.

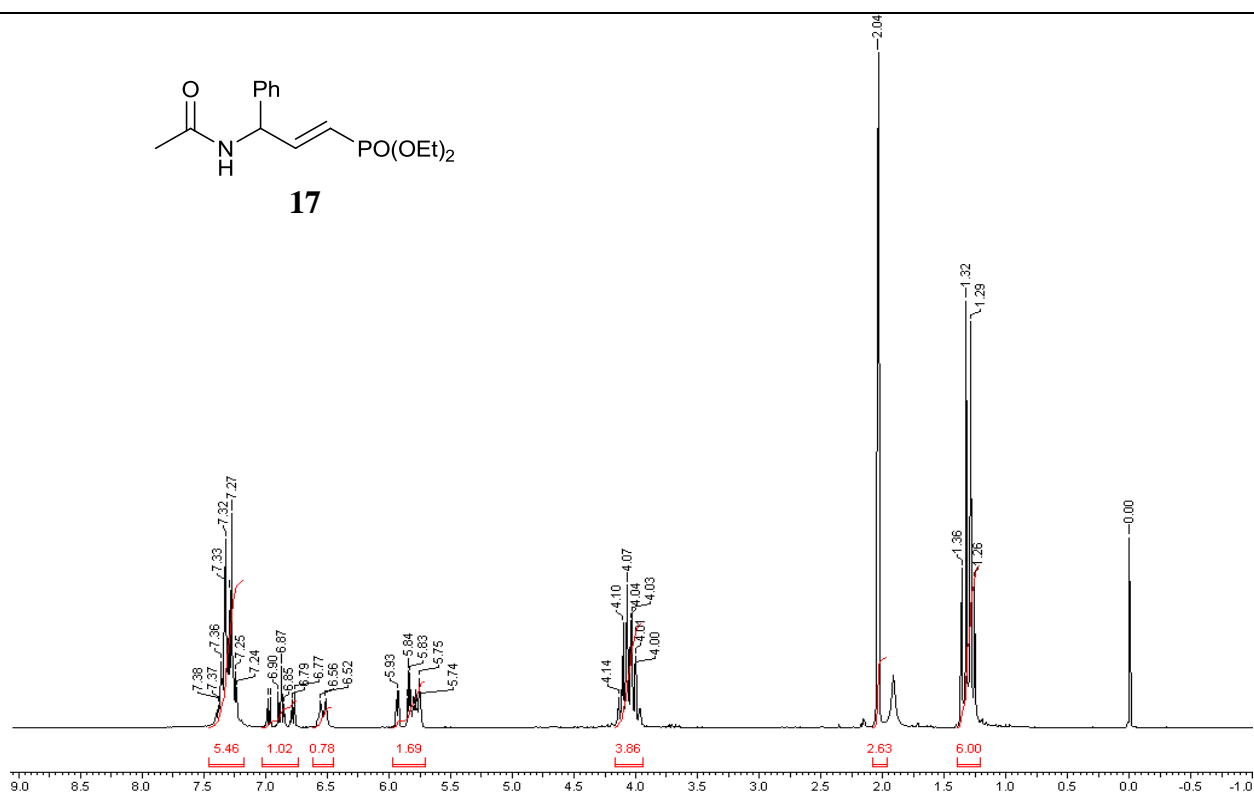
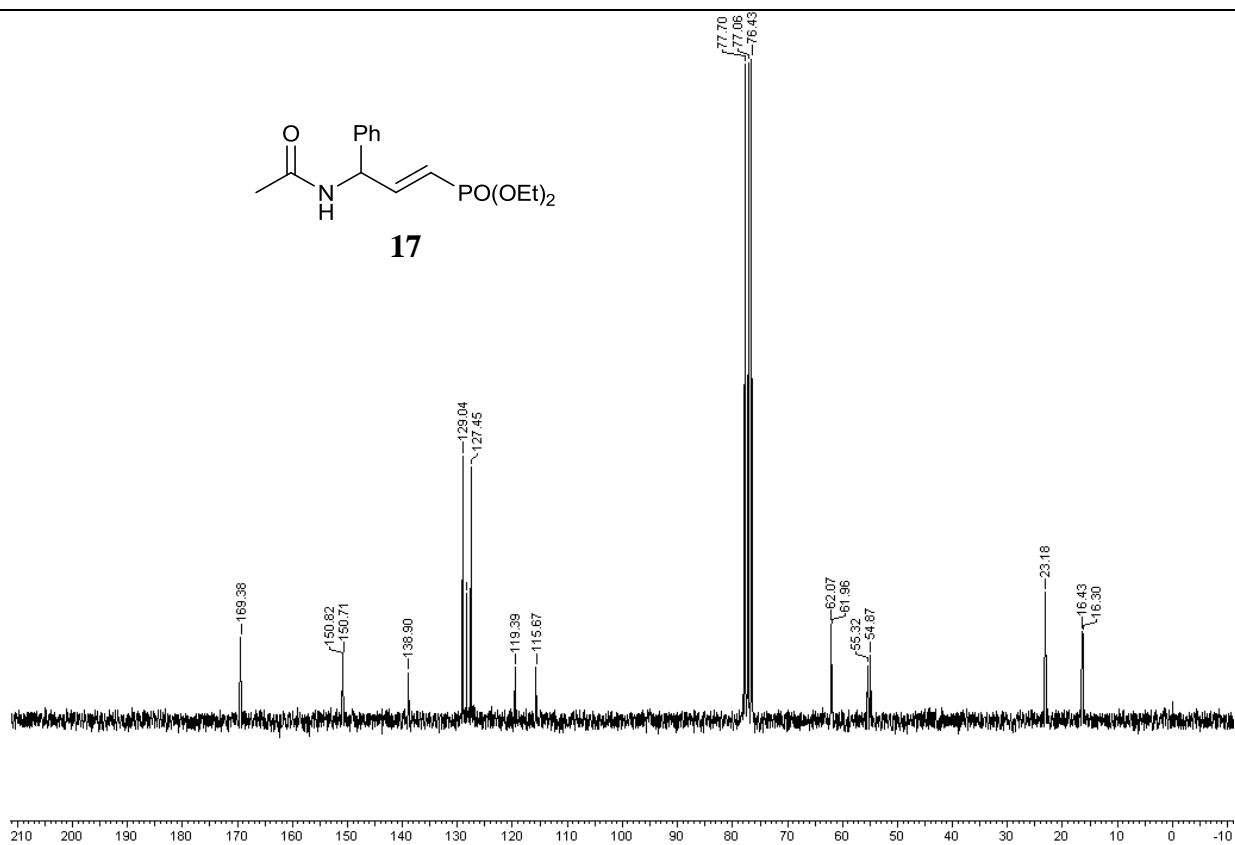
## Spectra

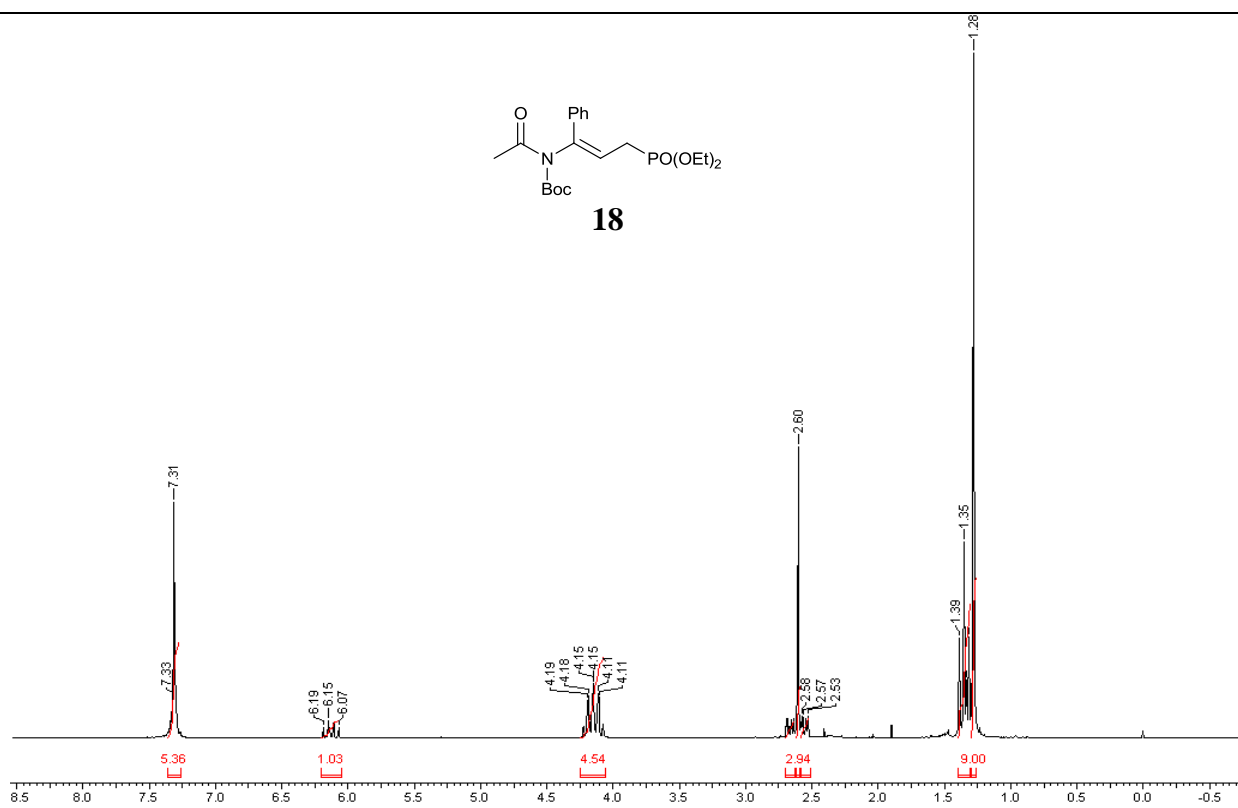
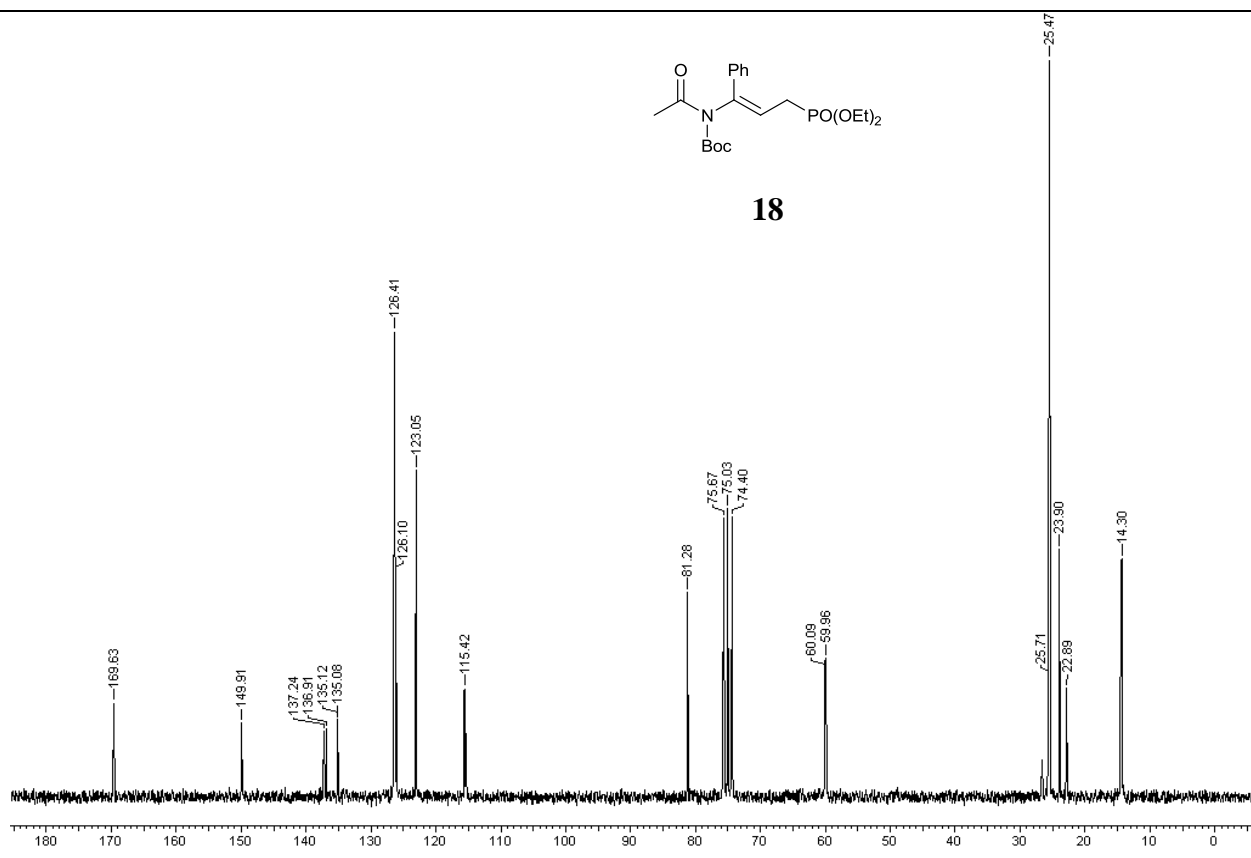
<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **8**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **8**

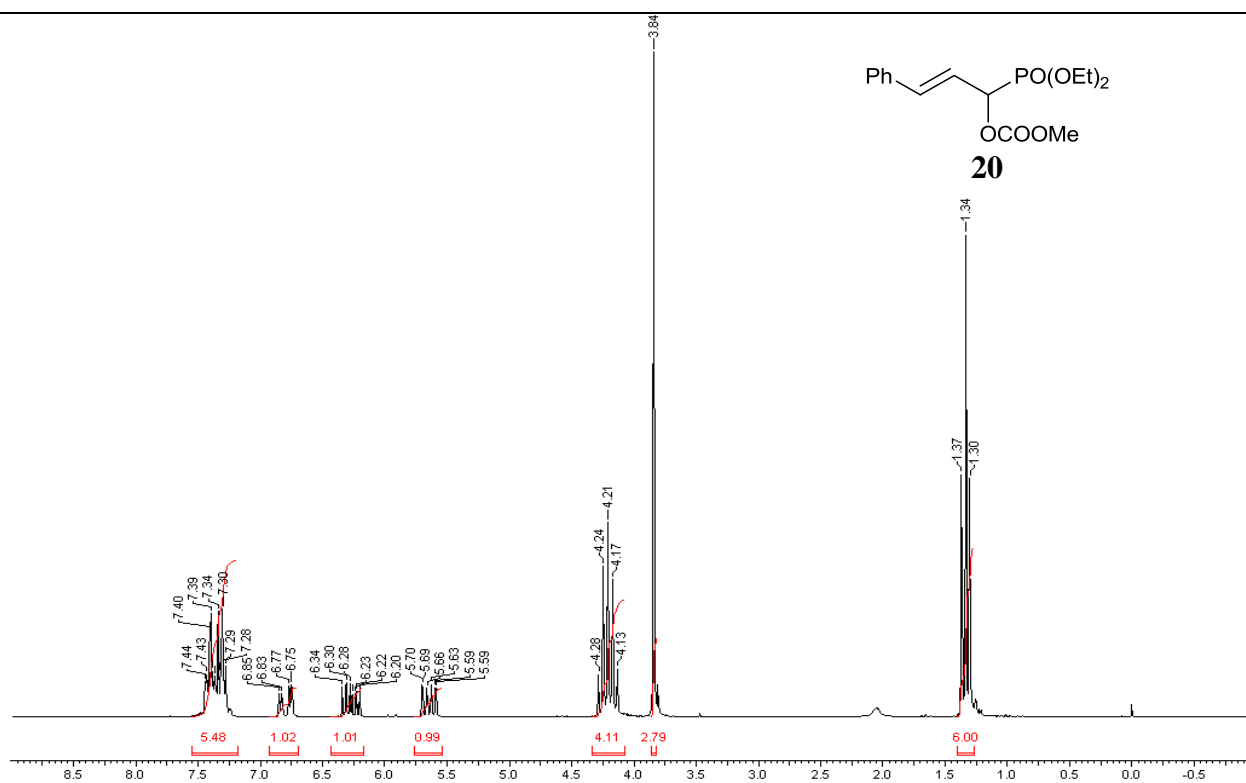
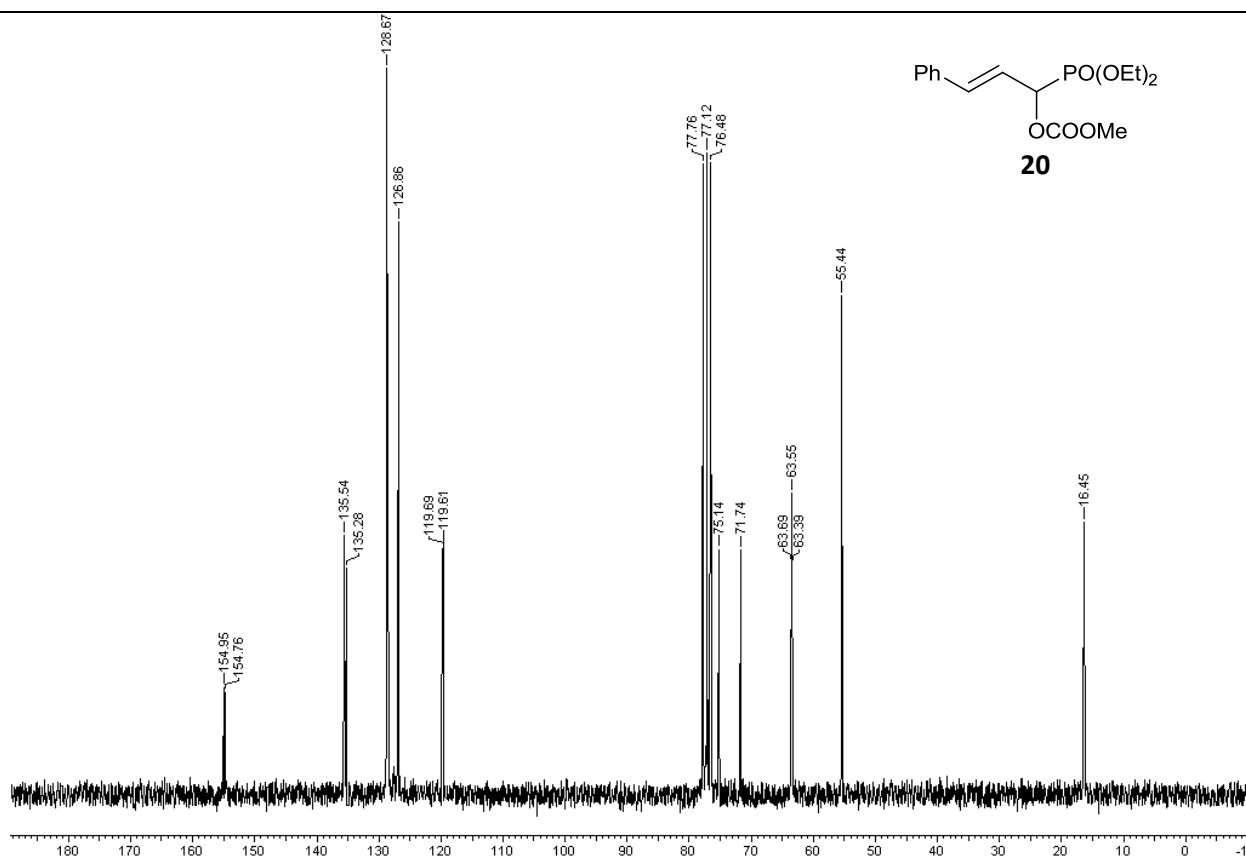
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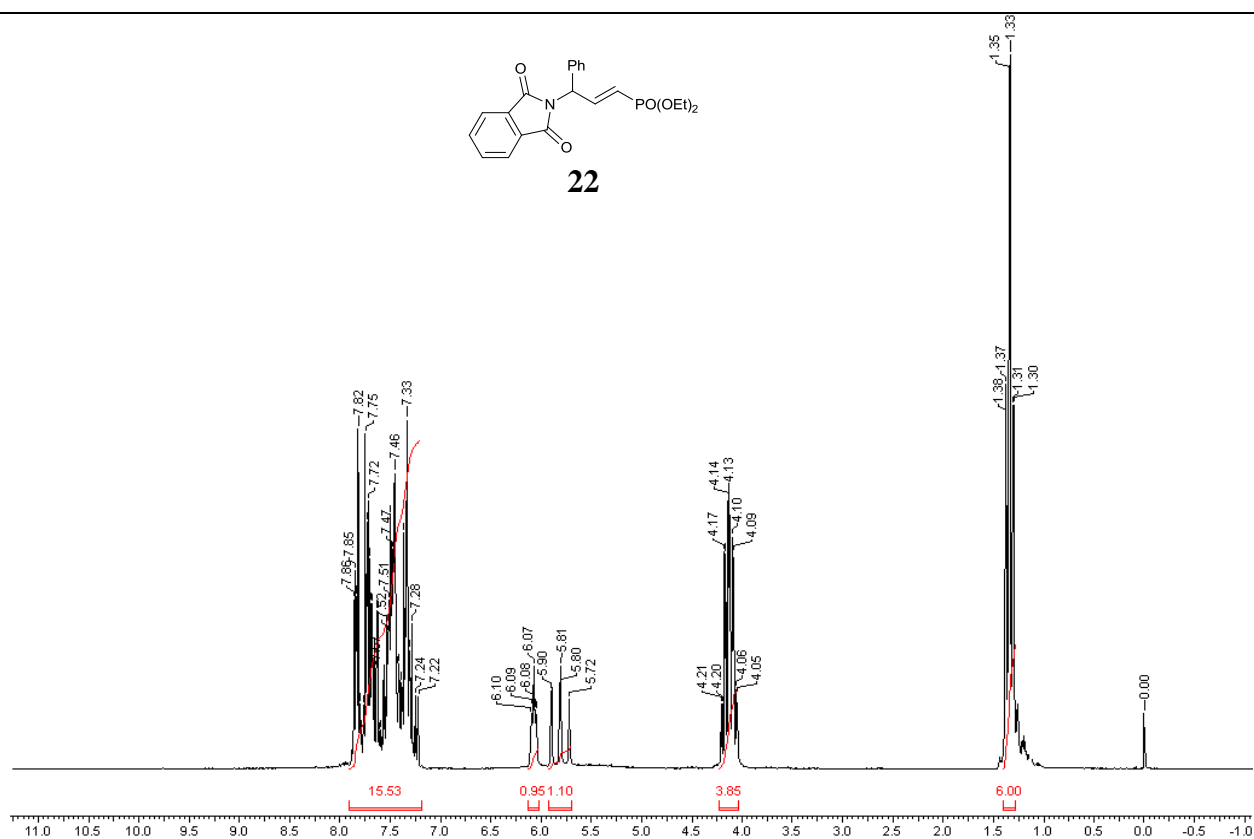
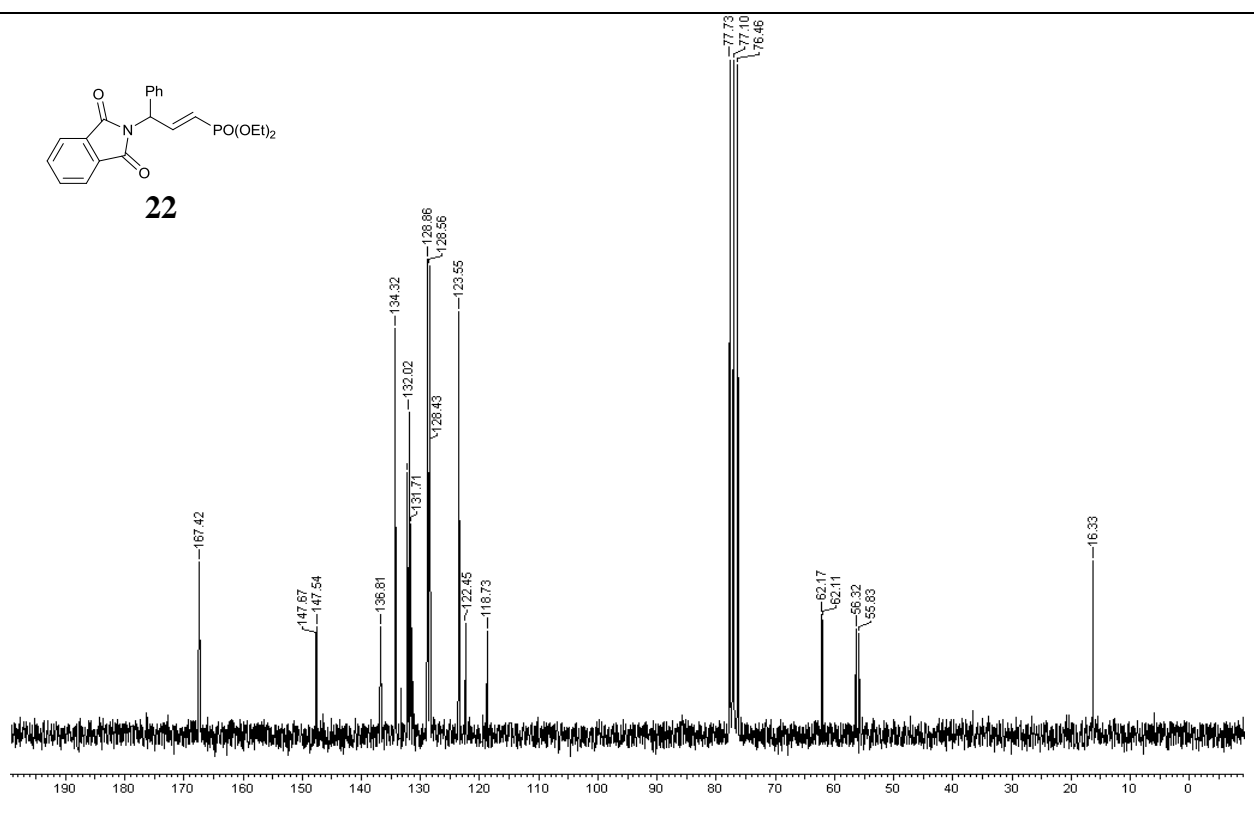
$^1\text{H}$  NMR (200 MHz, acetone- $d_6$  +  $\text{CDCl}_3$ ) of **14** $^{13}\text{C}$  NMR (50 MHz, acetone- $d_6$  +  $\text{CDCl}_3$ ) of **14**

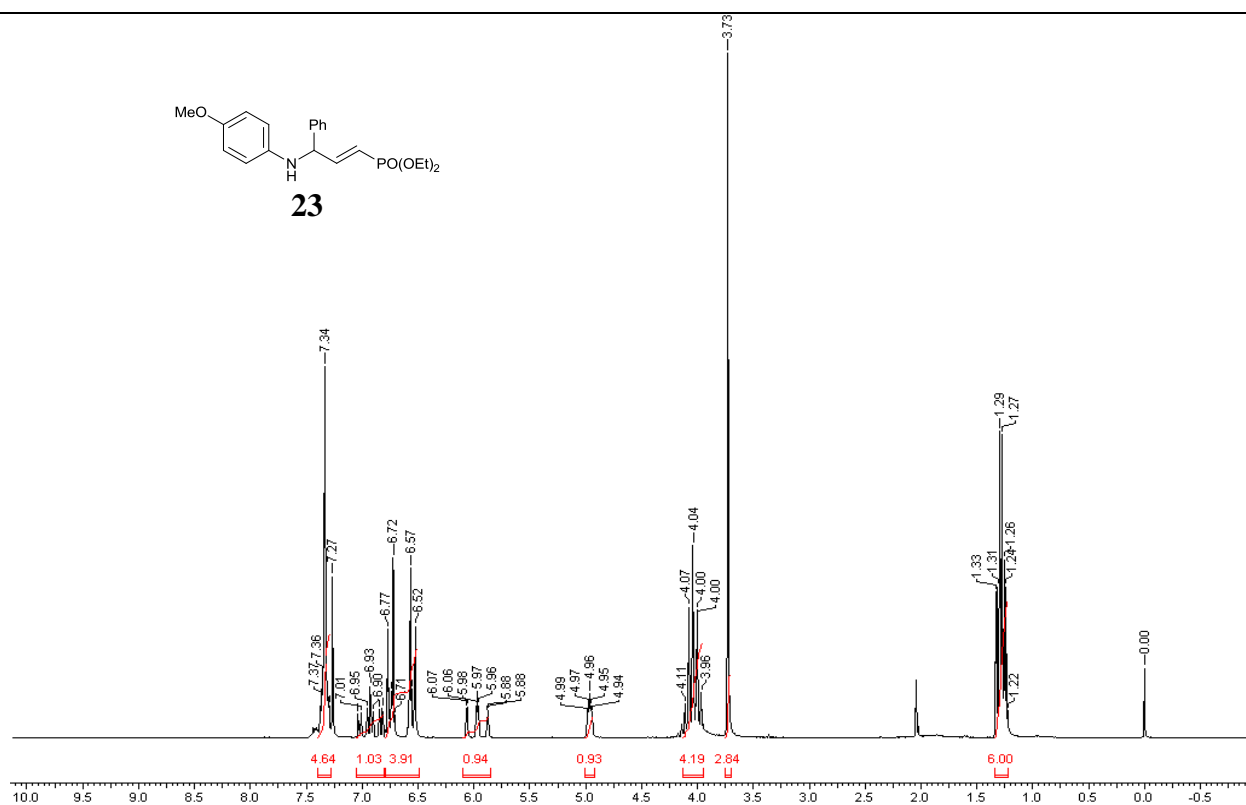
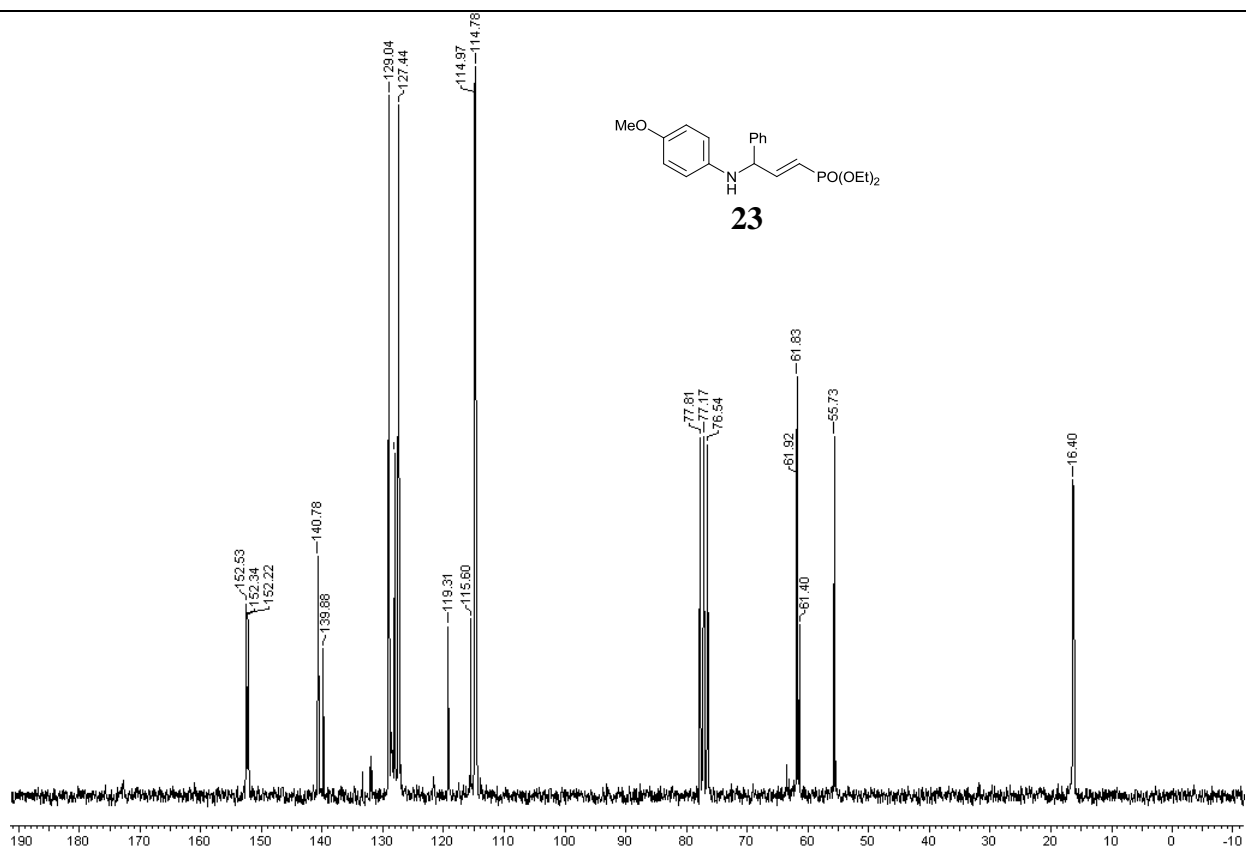


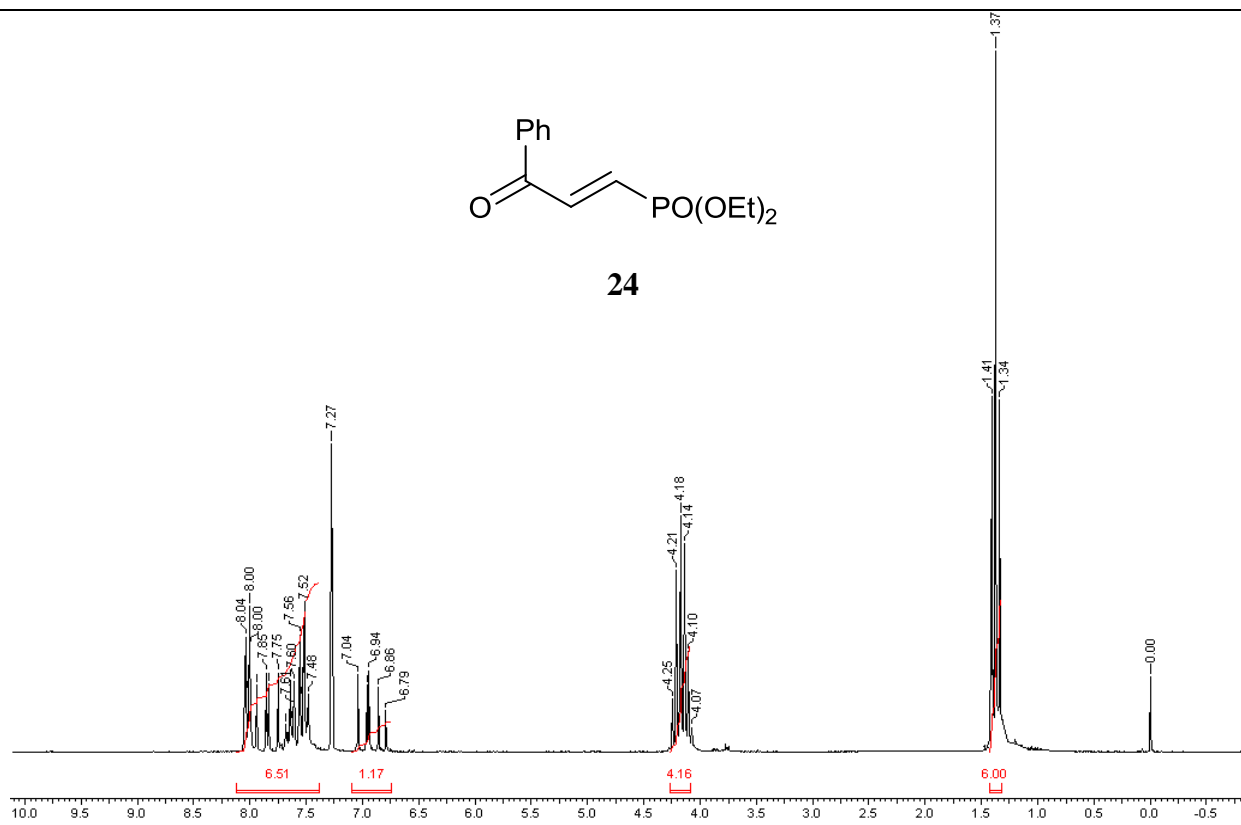
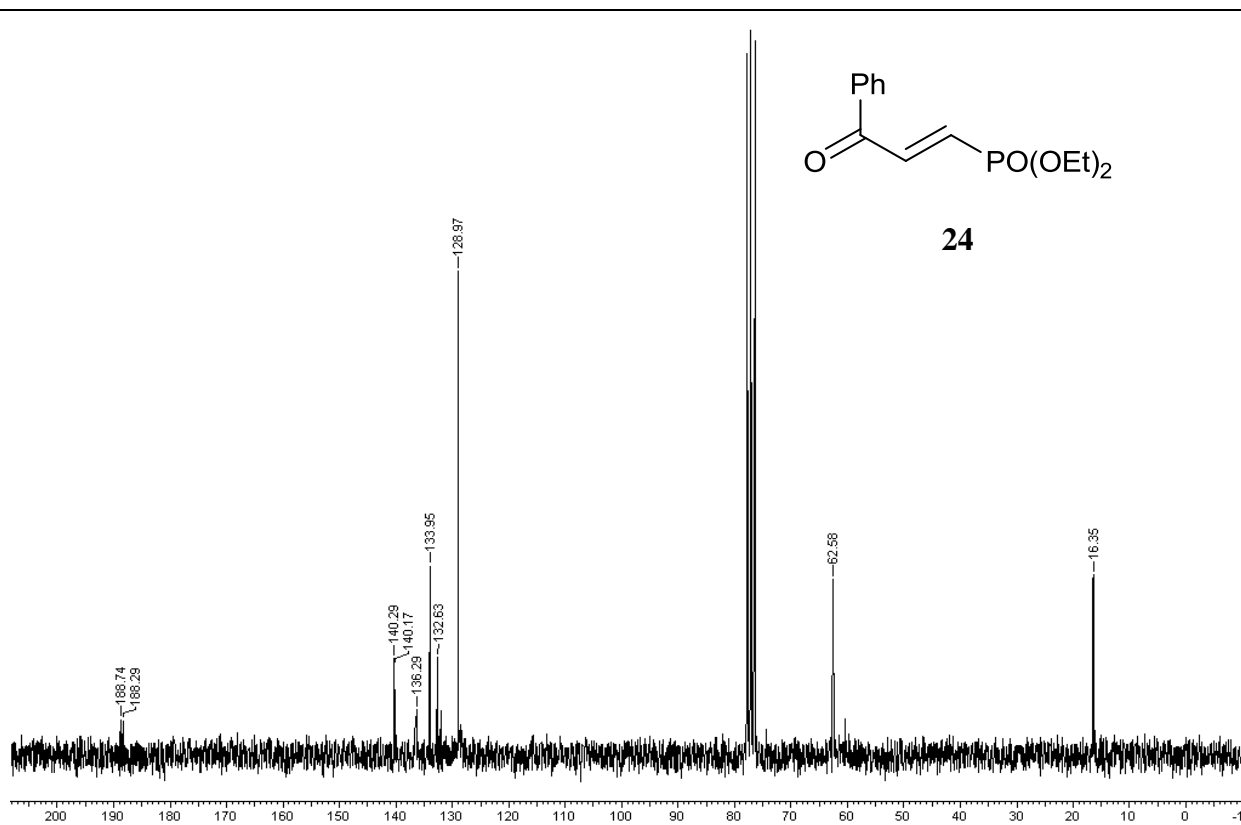
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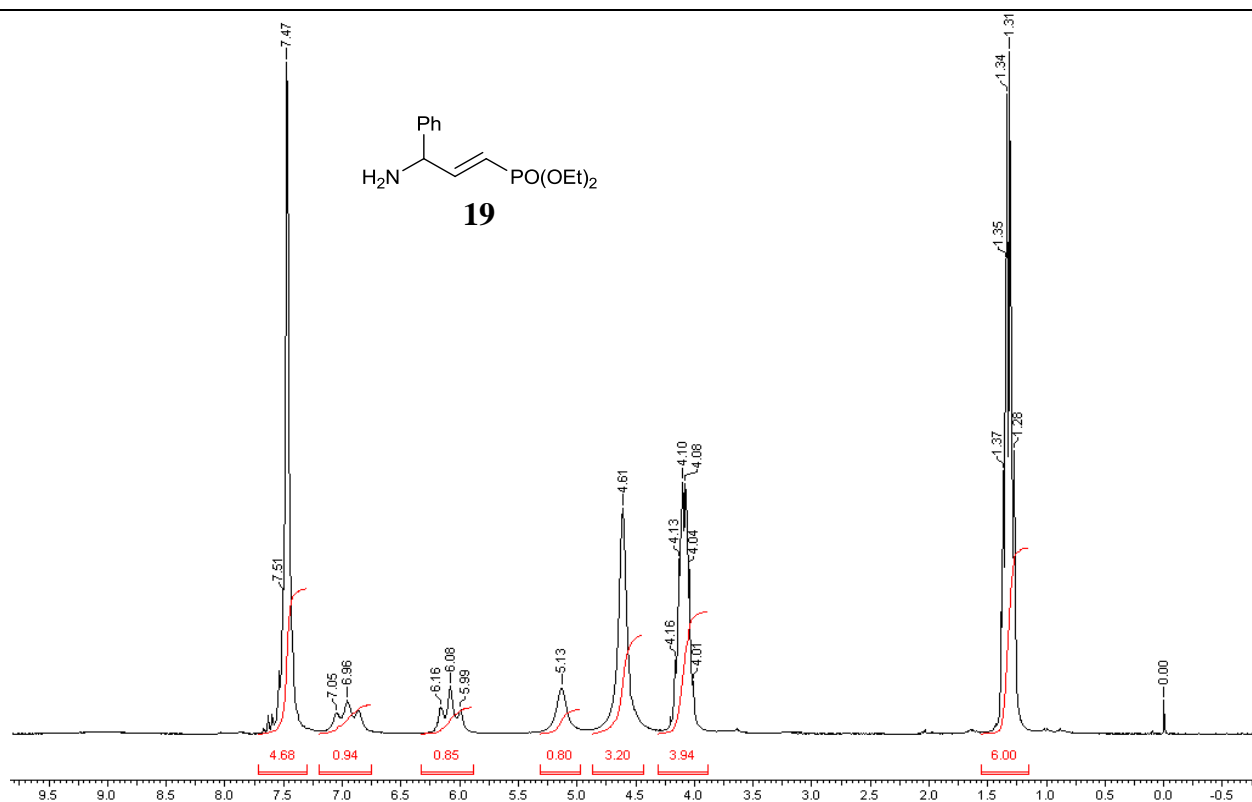
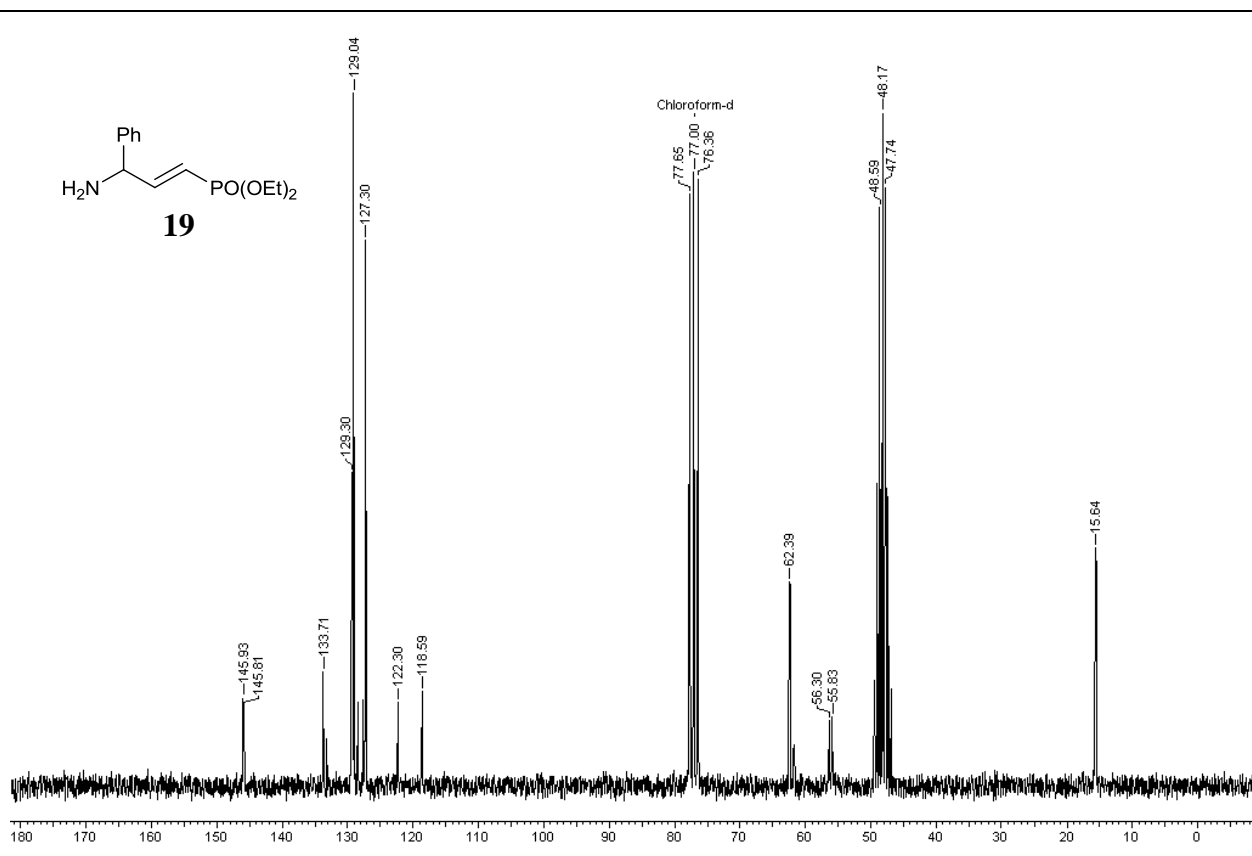
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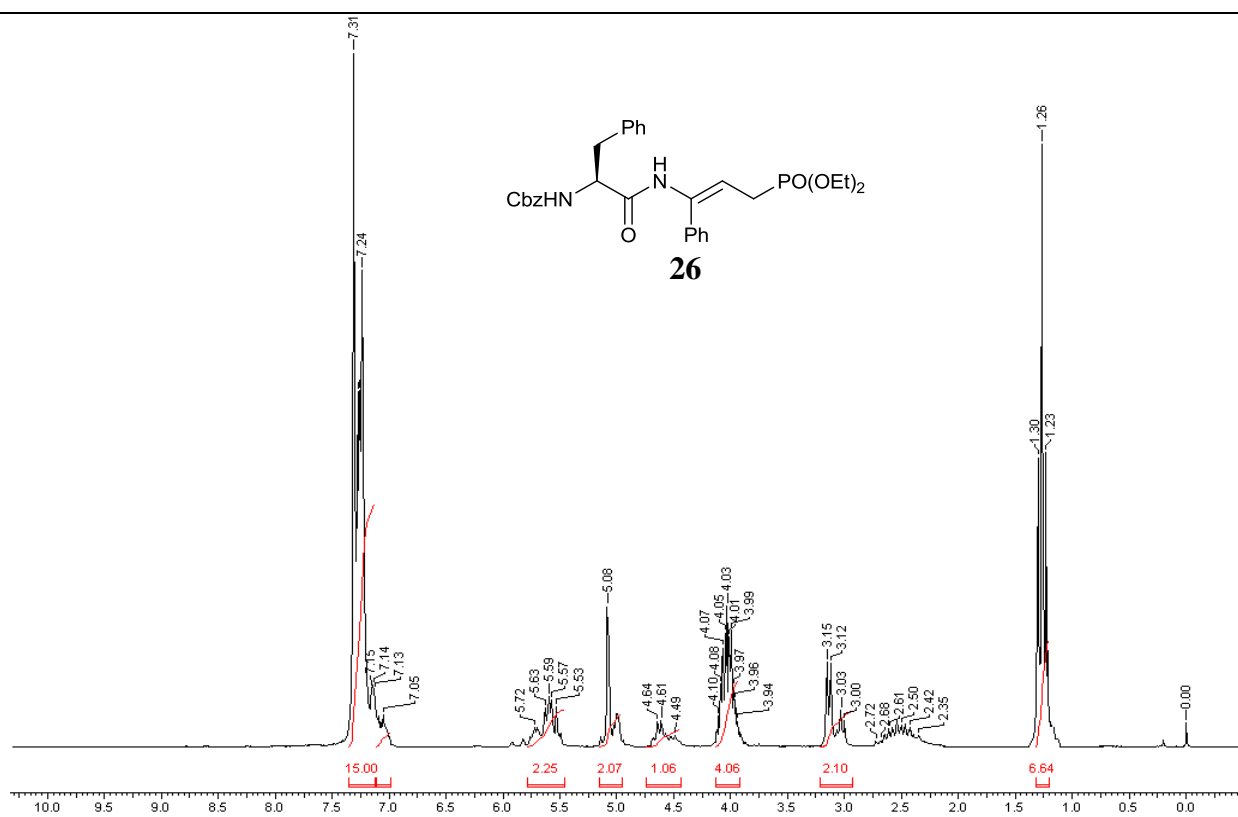
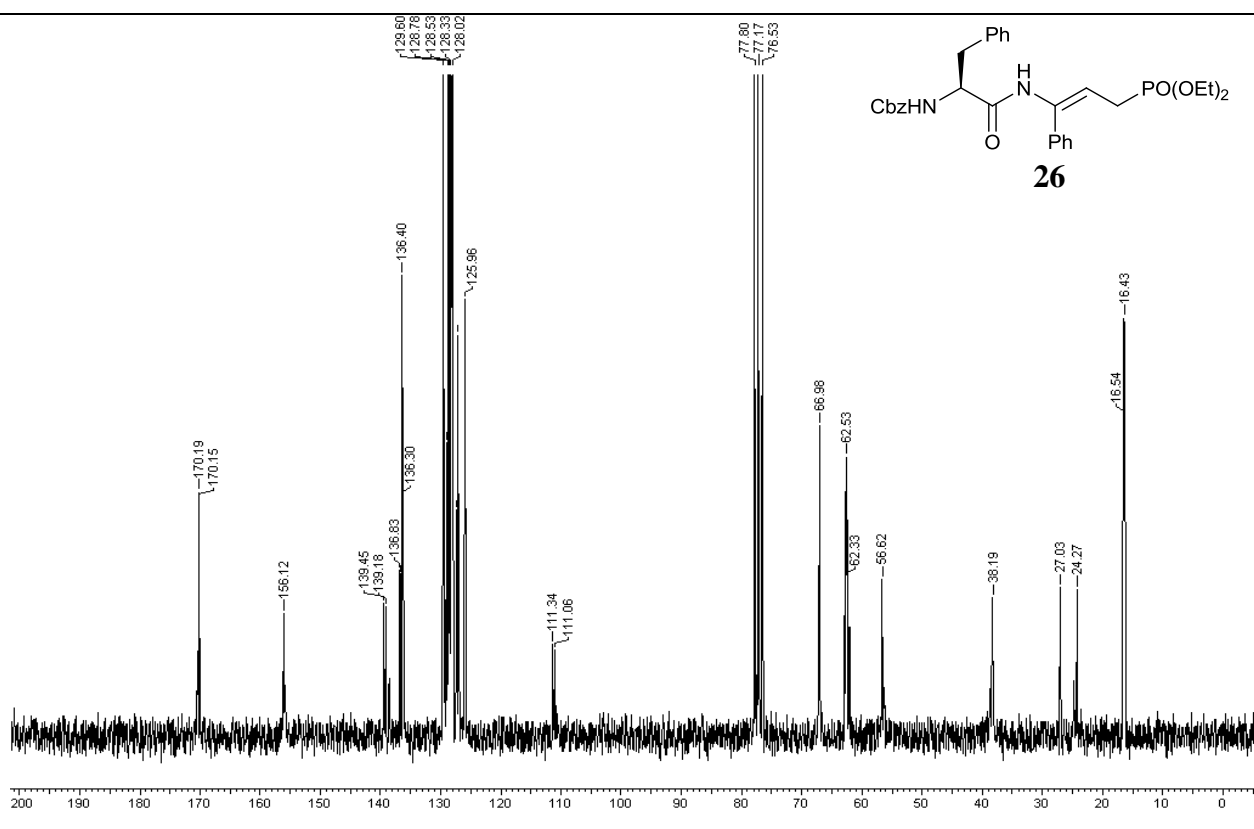
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$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **22** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **22**

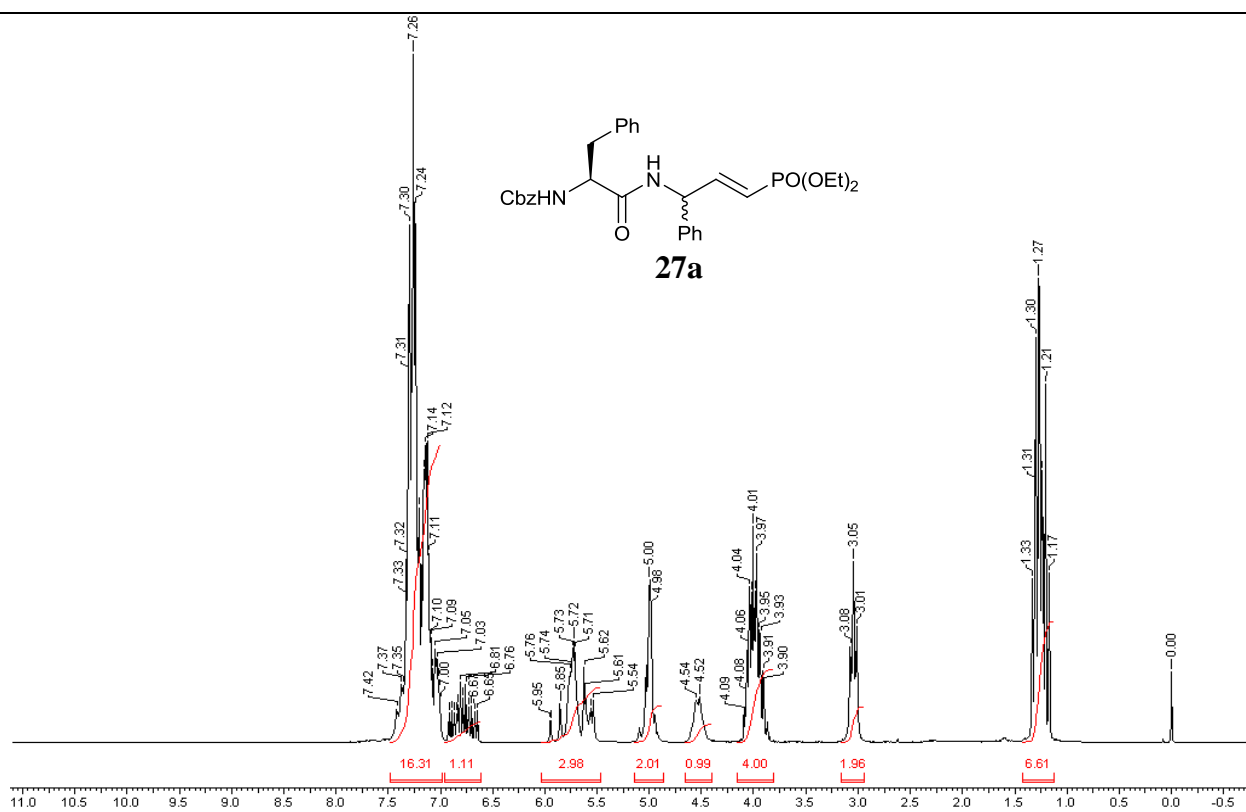
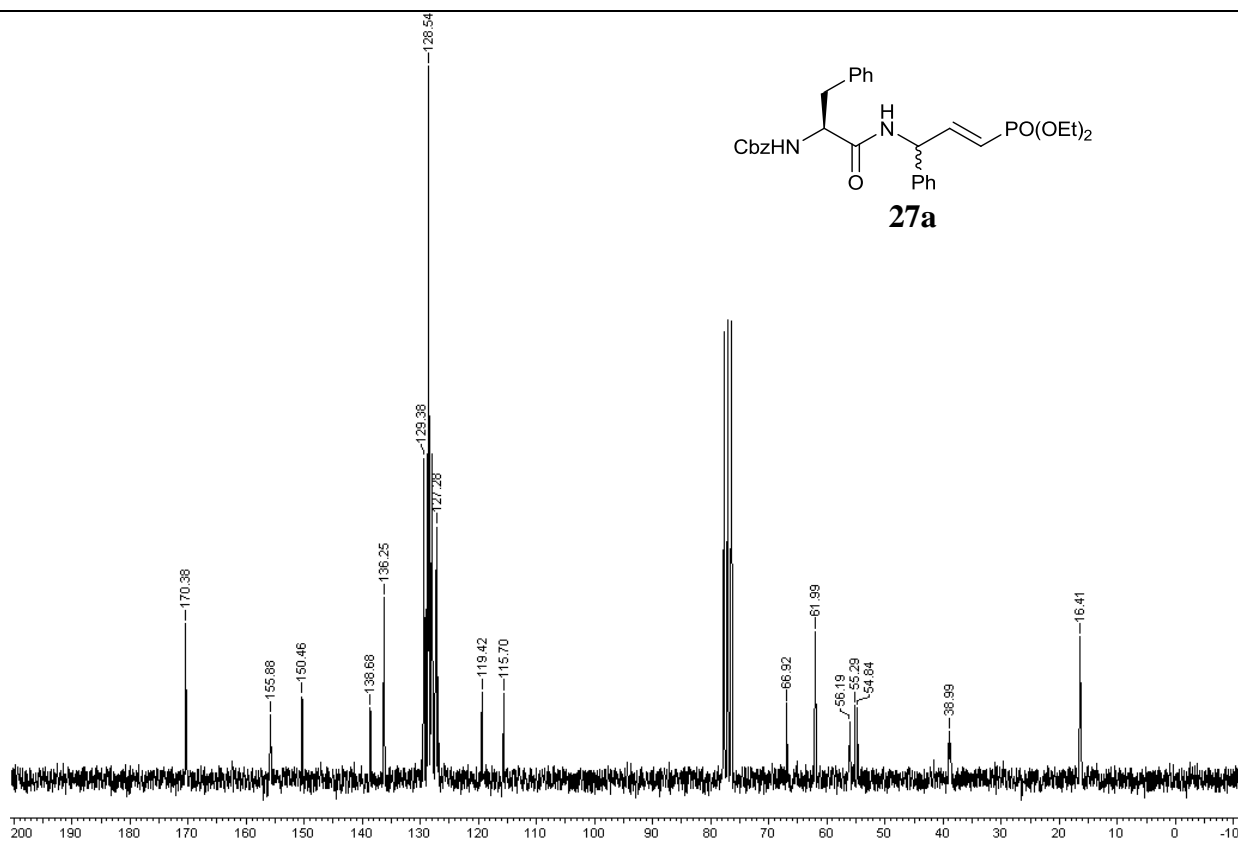
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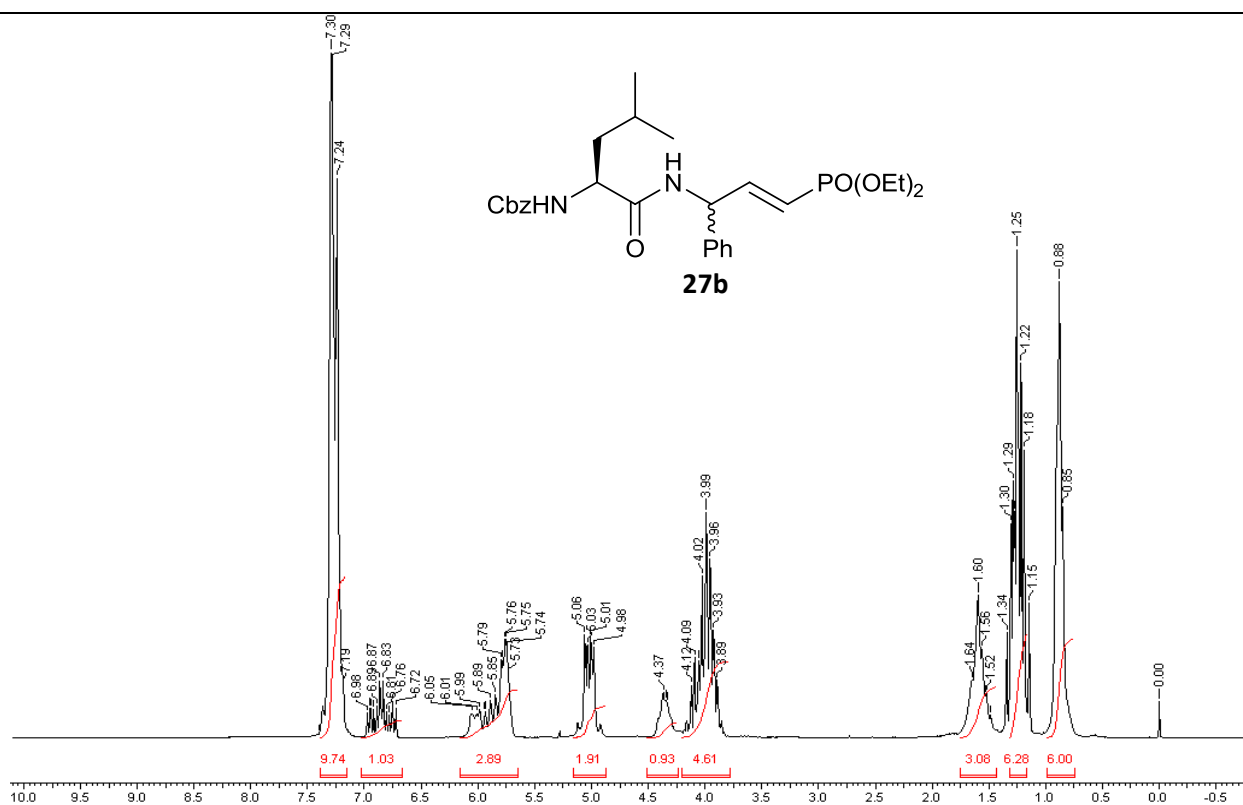
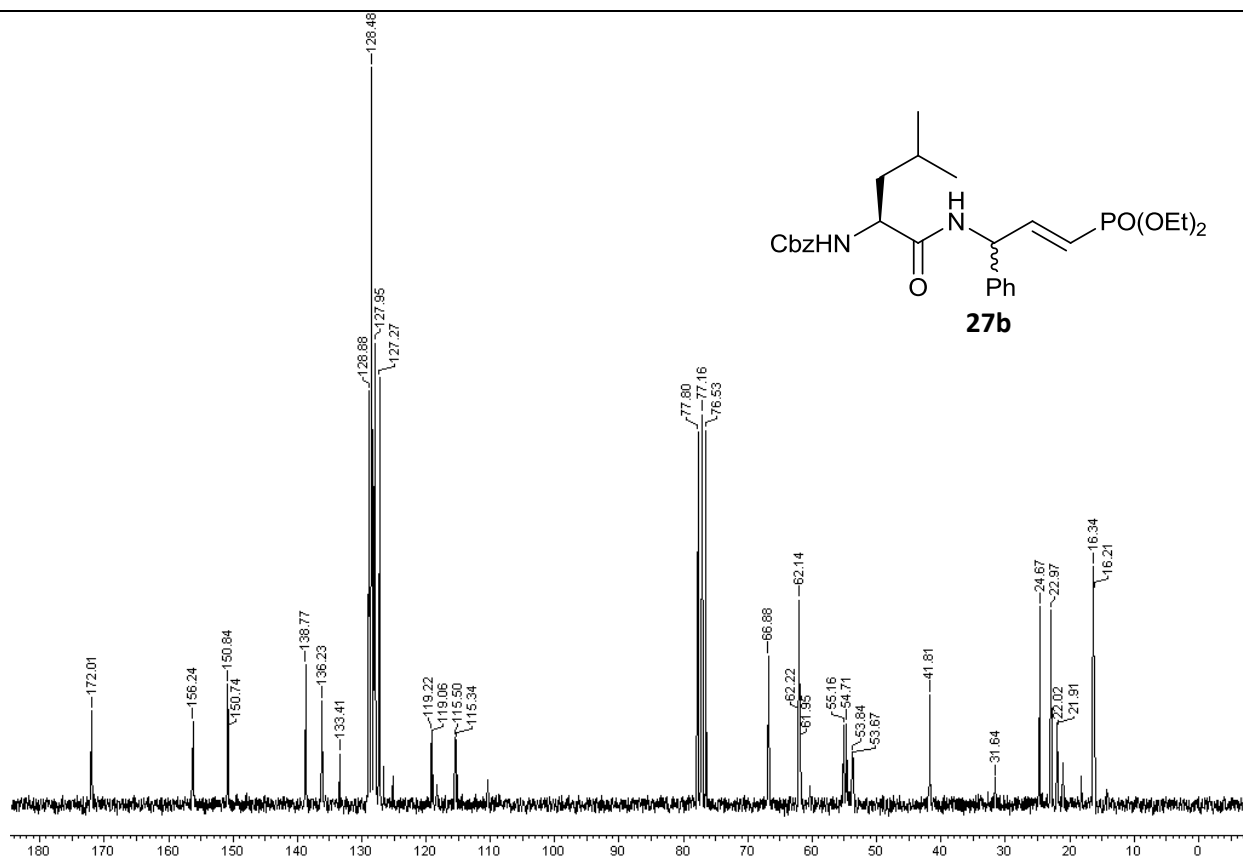
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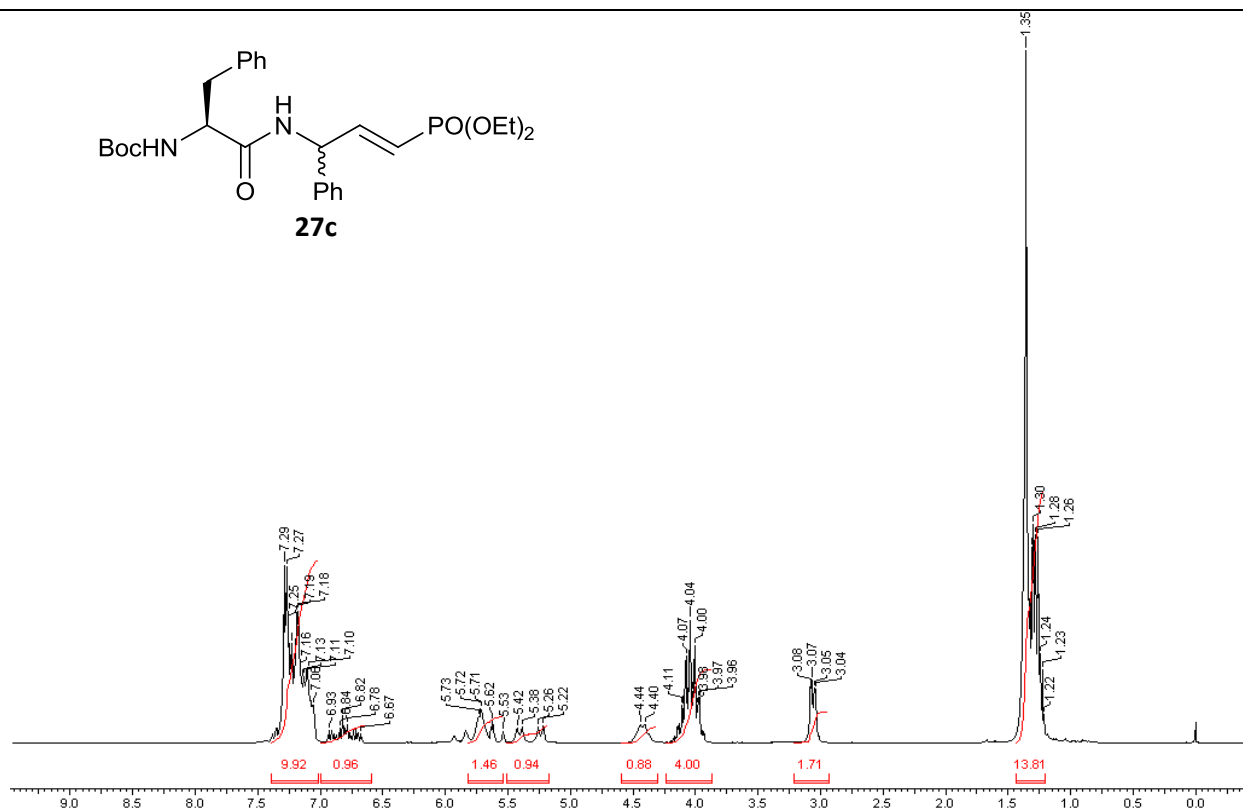
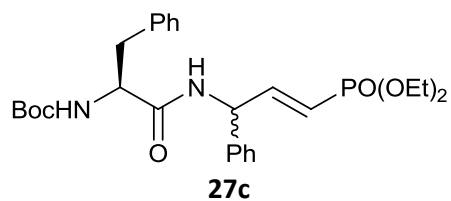
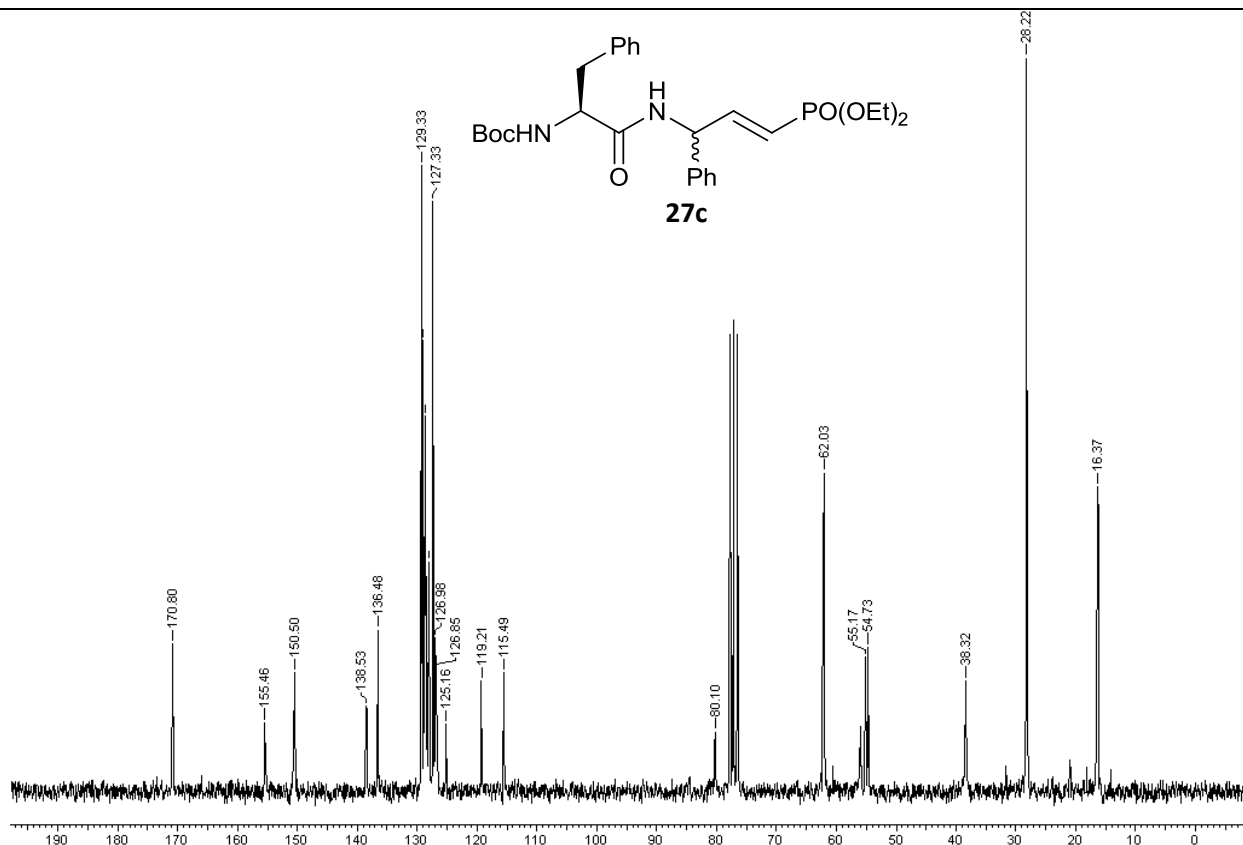
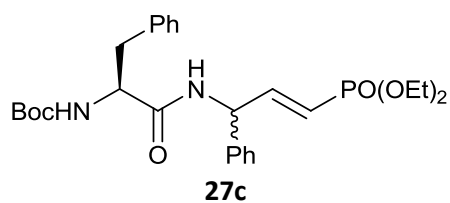
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **19** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **19**

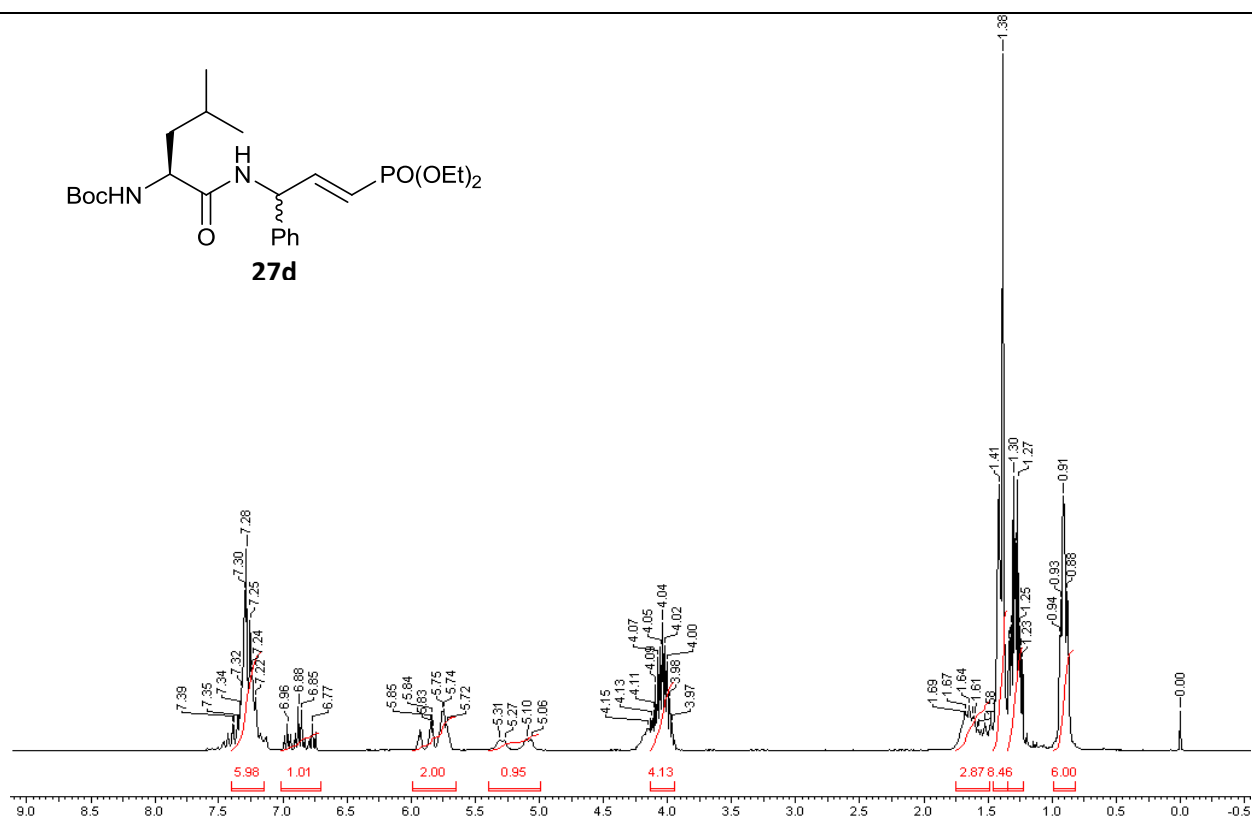
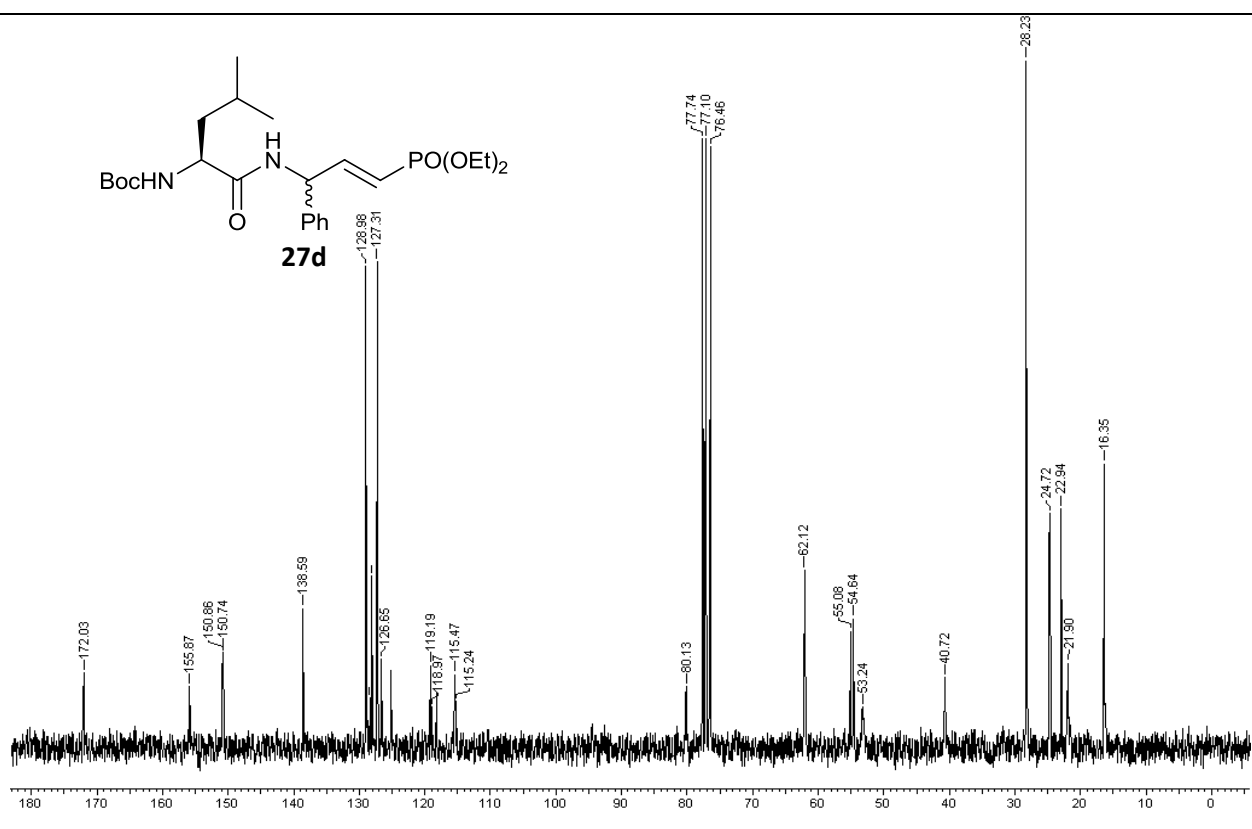
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **26** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **26**

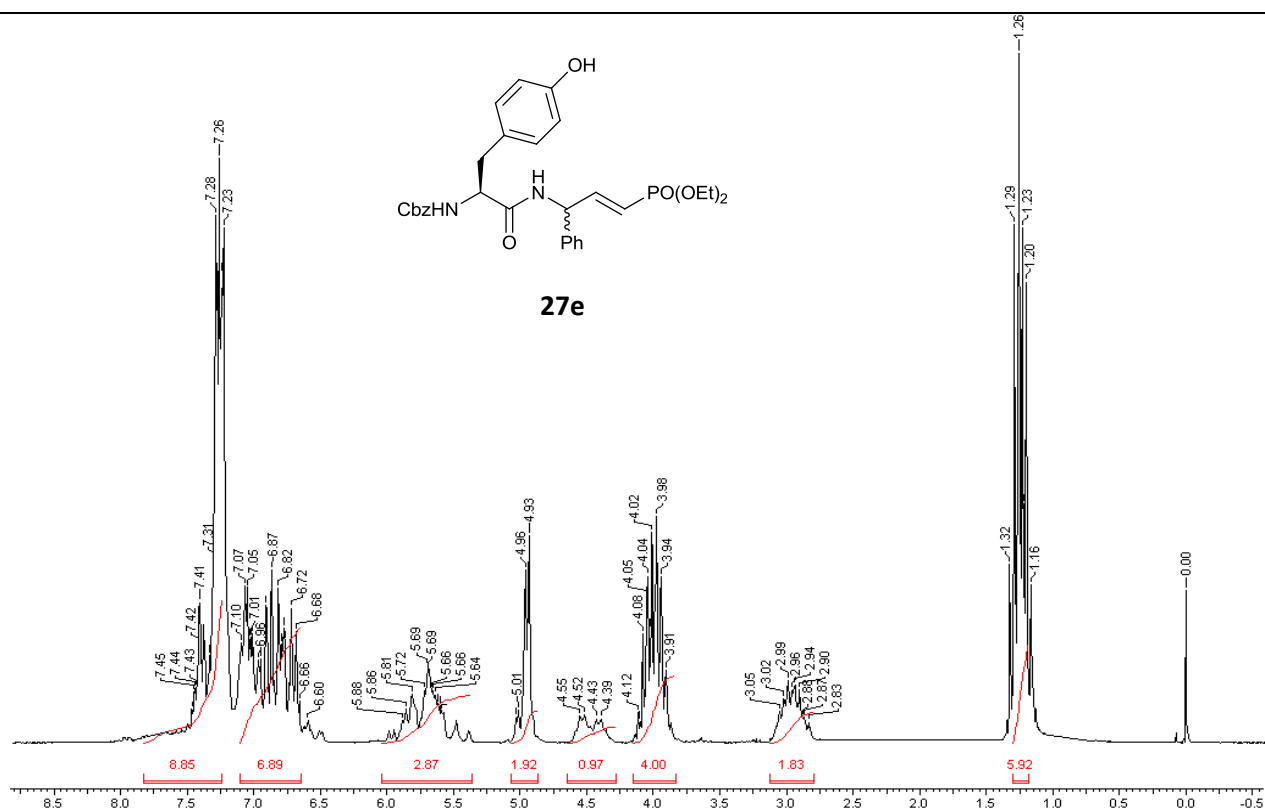
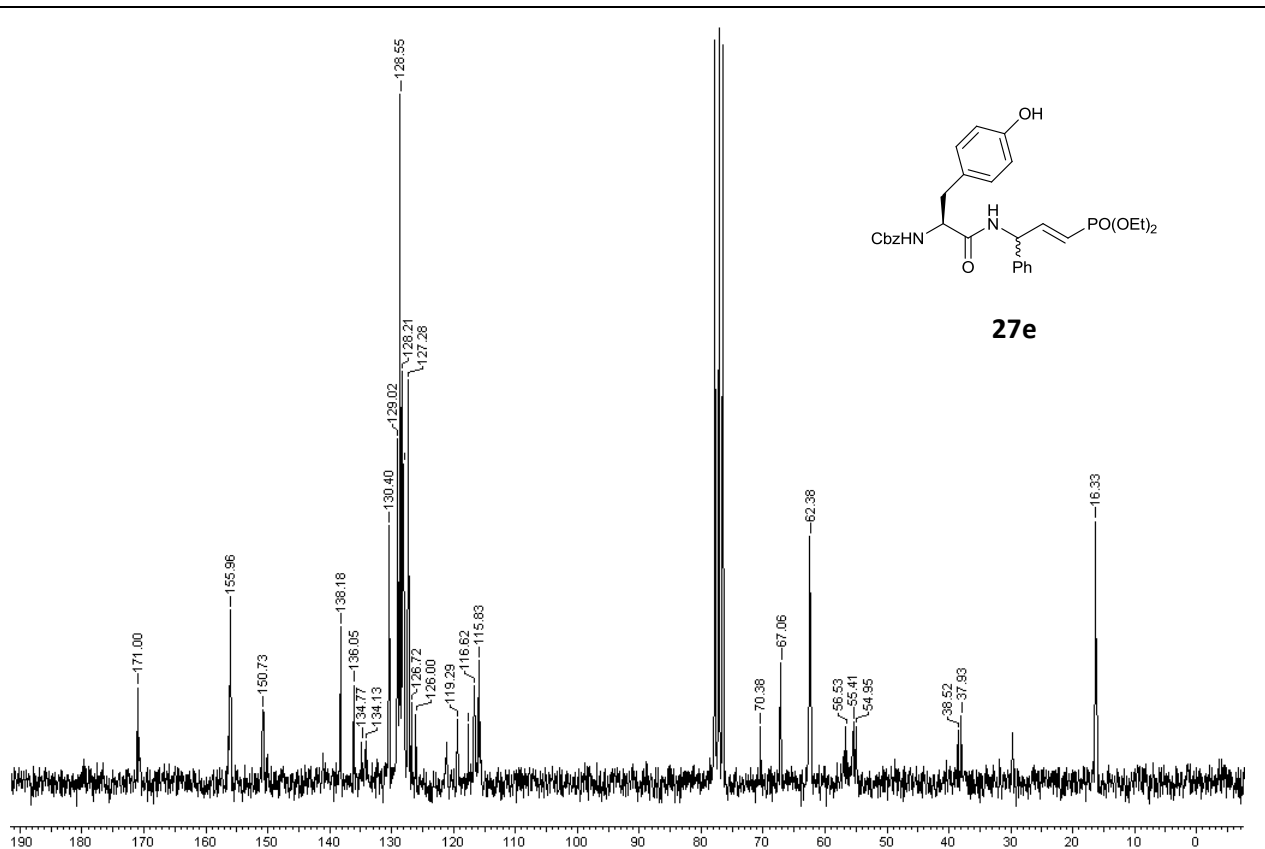


<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **27a**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **27a**

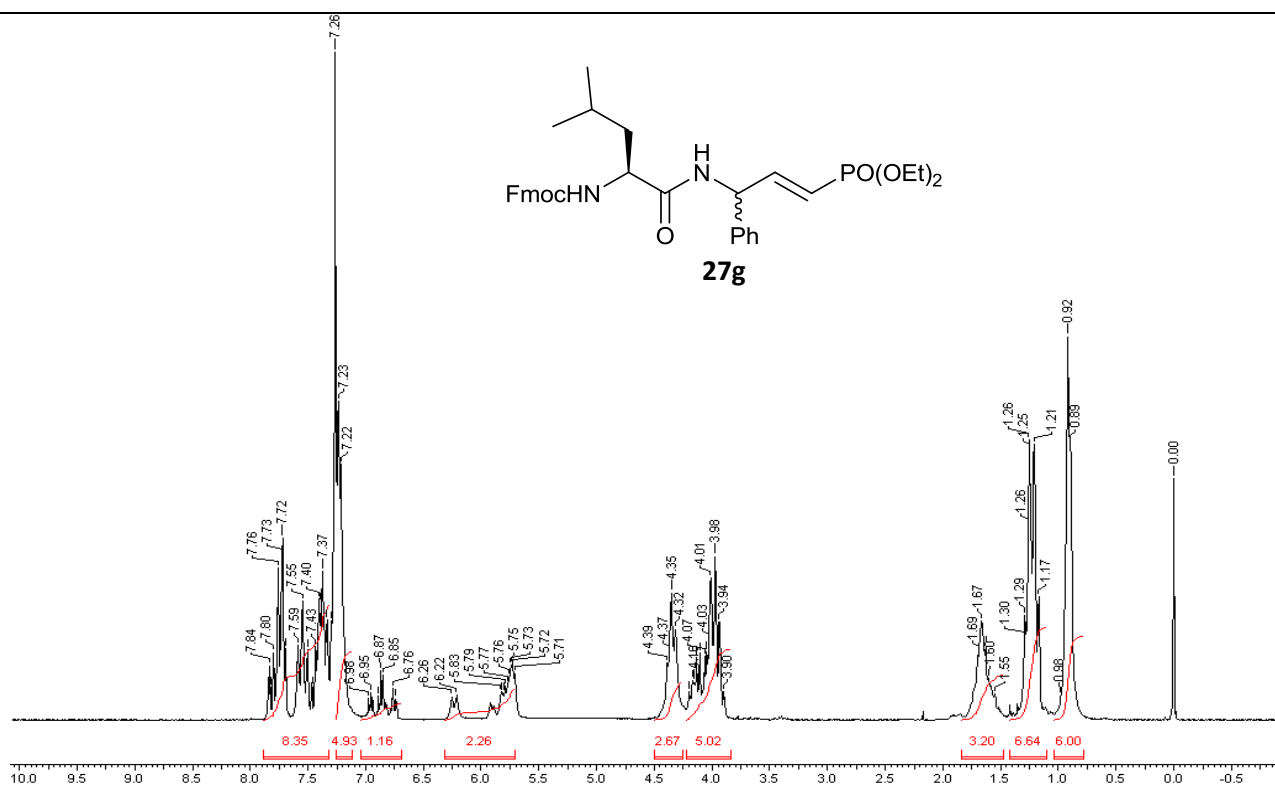
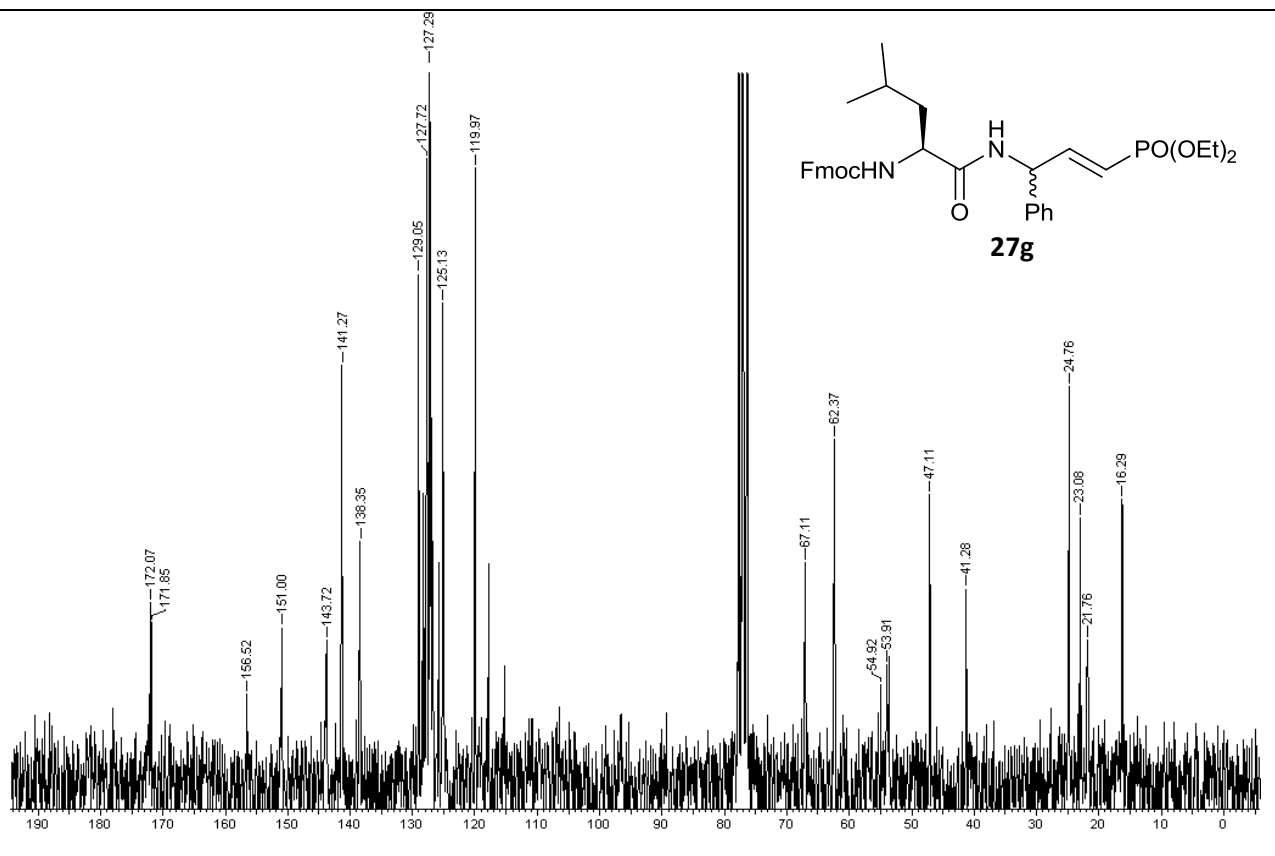
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **27b** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **27b**

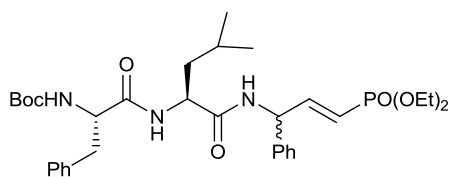
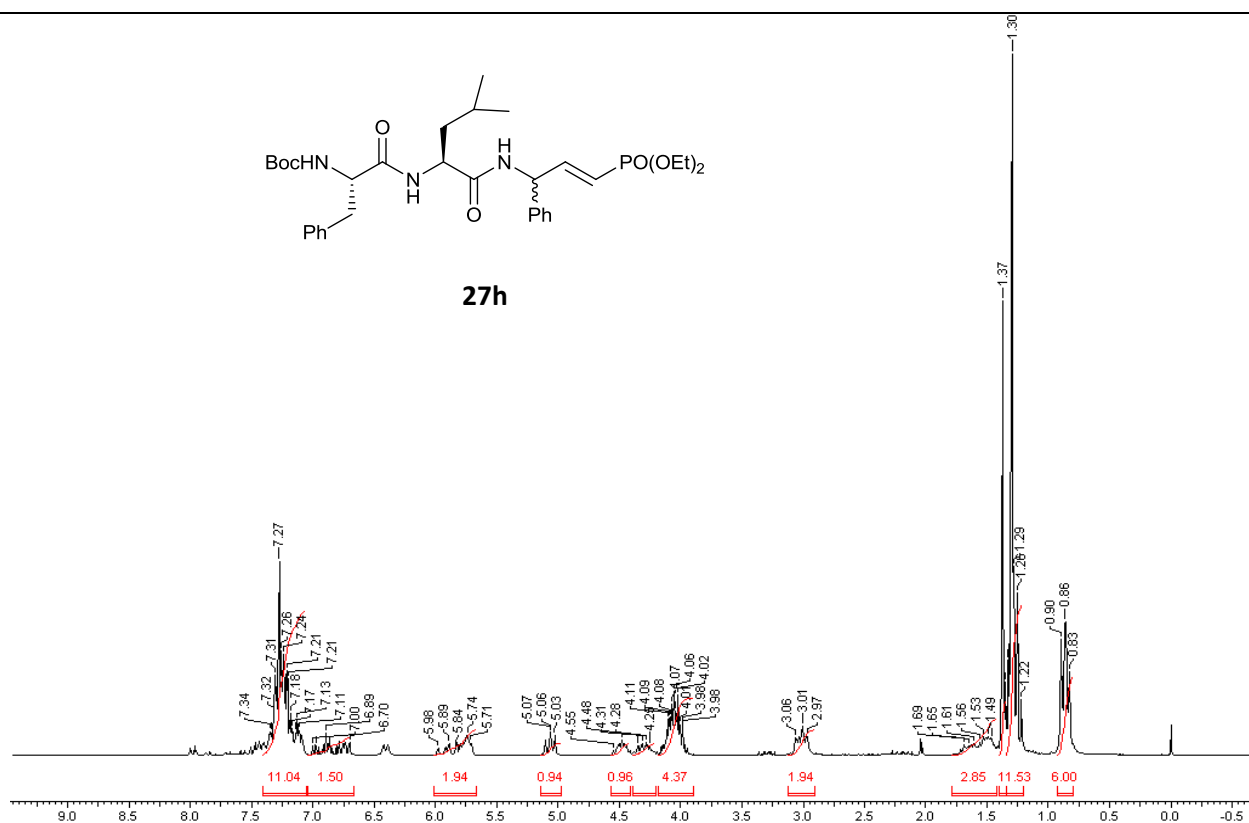
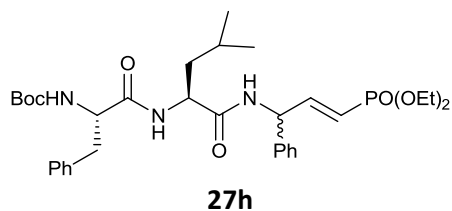
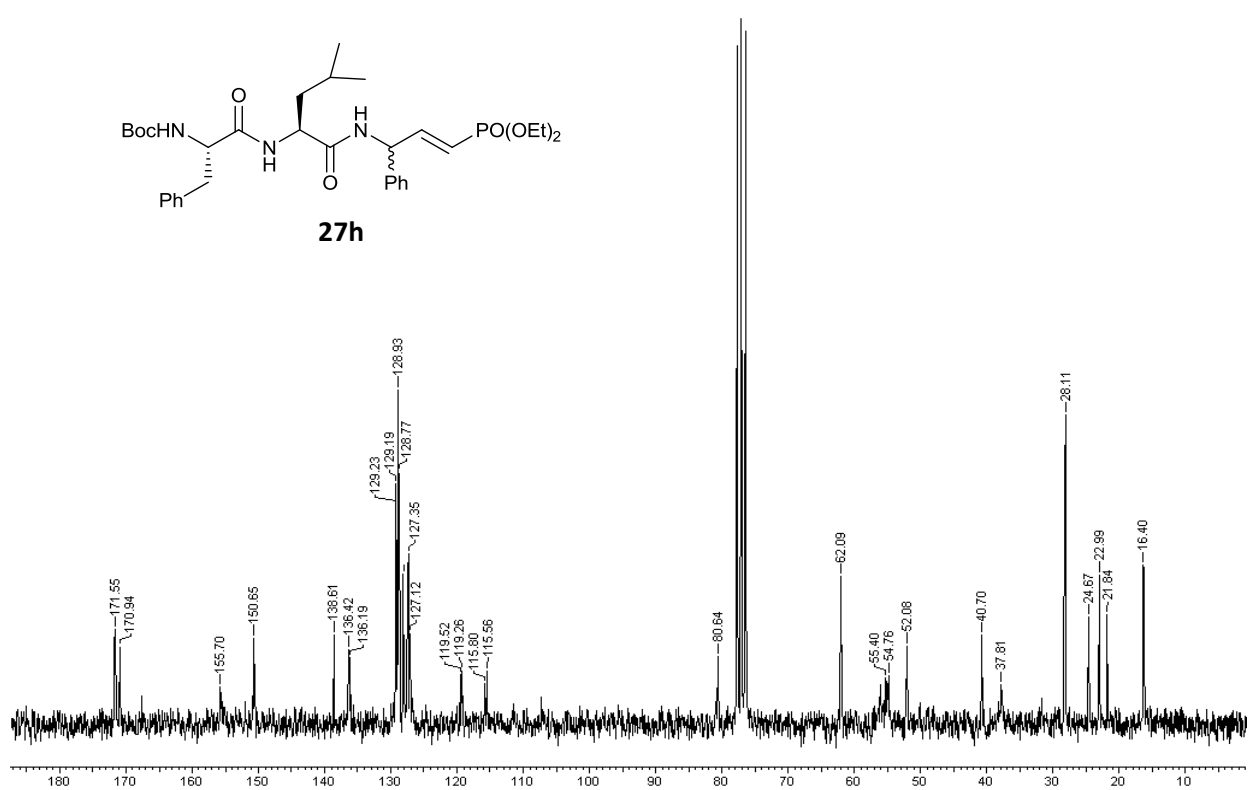
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **27c** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **27c**

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **27d** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **27d**

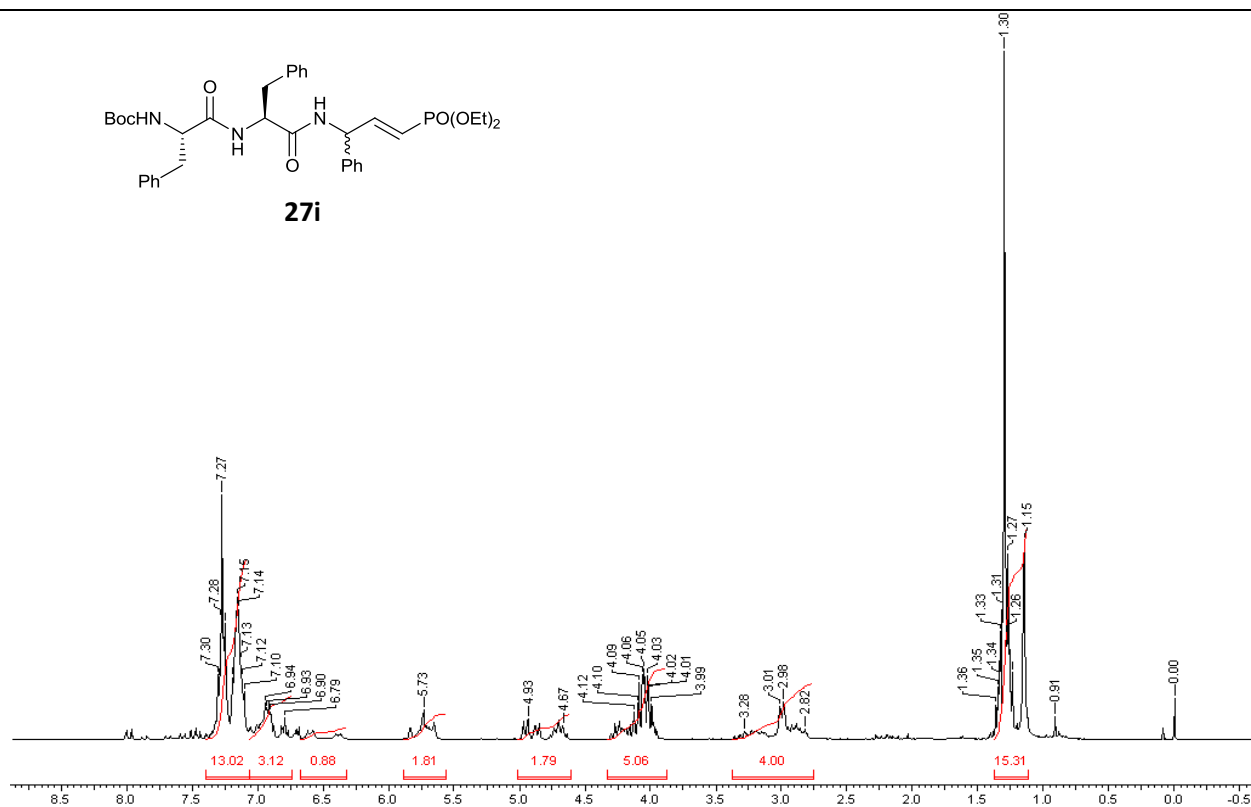
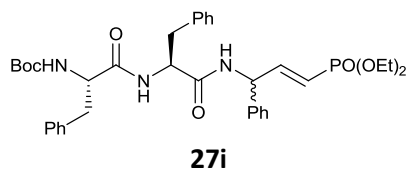
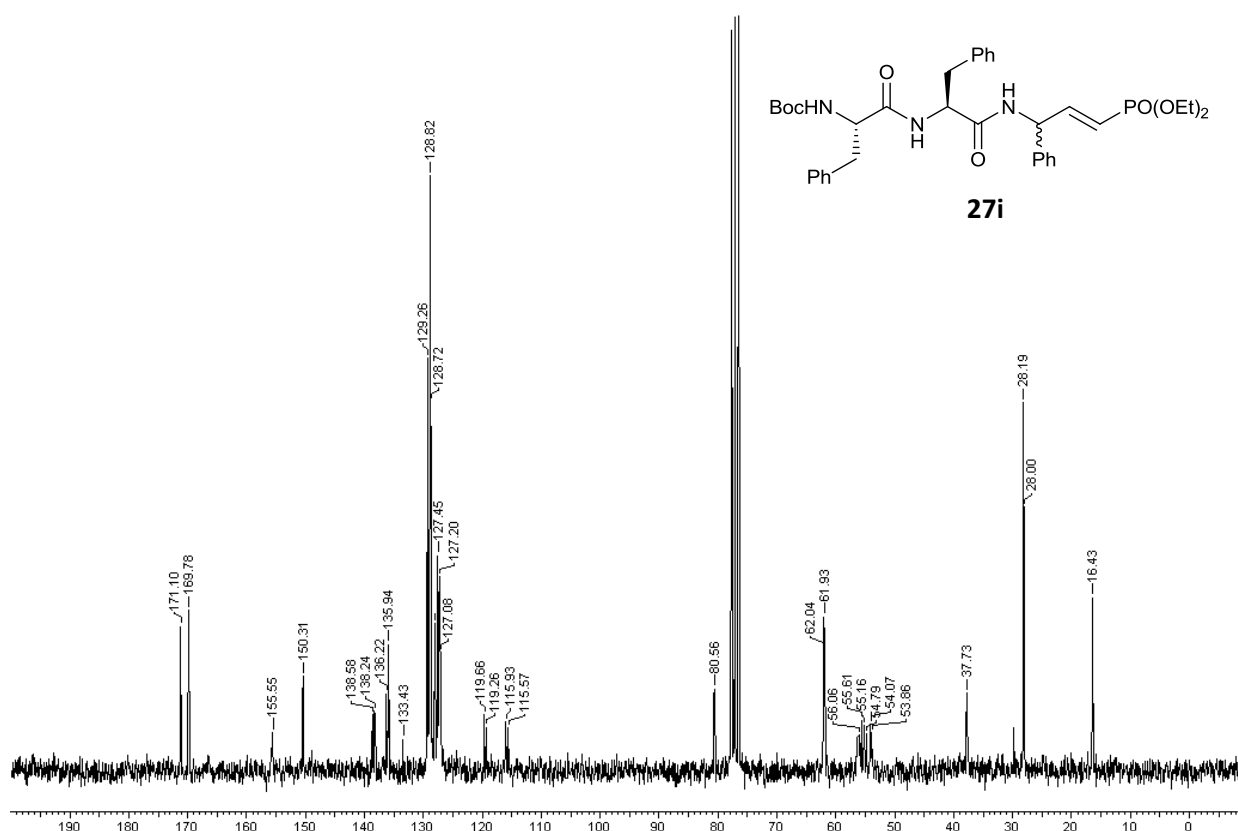
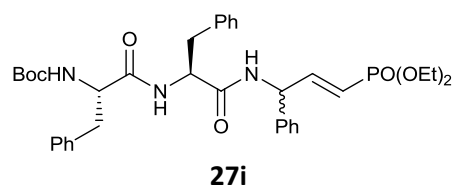
<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **27e**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **27e**

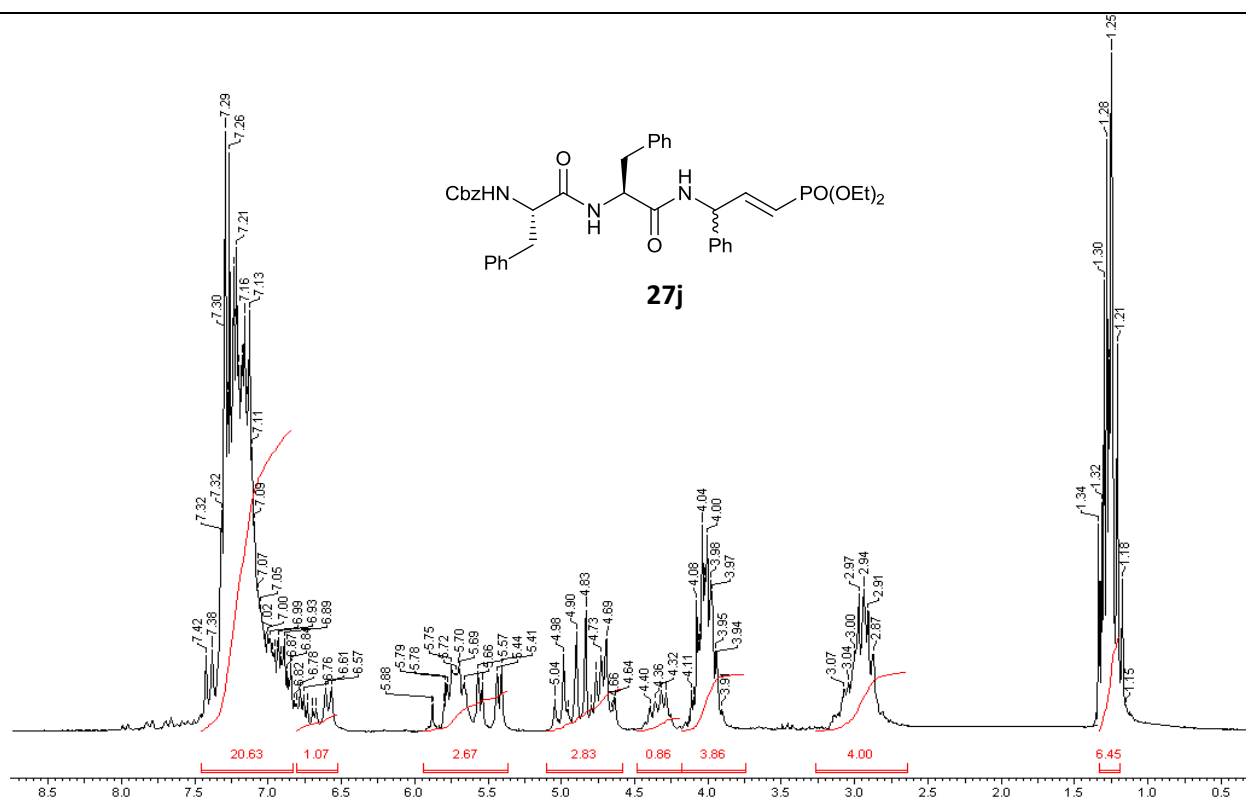
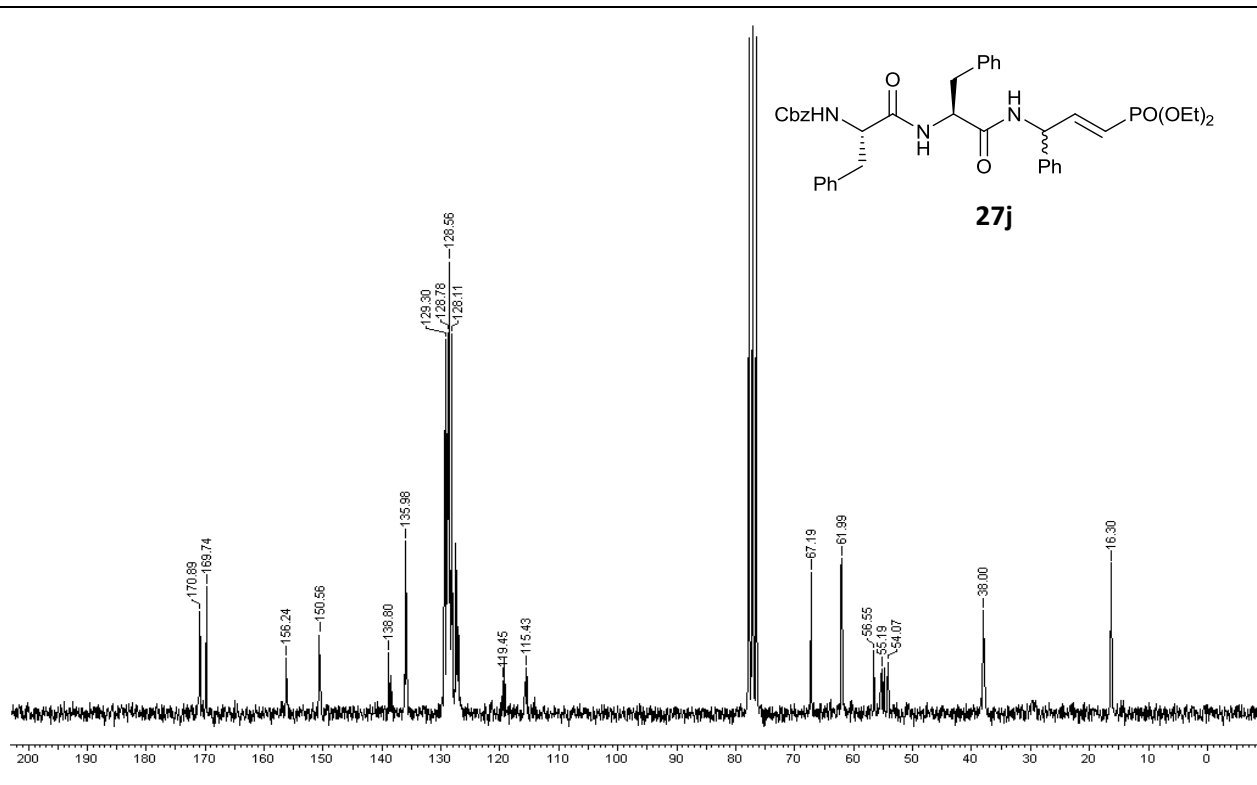


$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **27g** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **27g**

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **27h****27h**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **27h****27h**



$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **27i** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **27i**

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **27j**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **27j**

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19. Following techniques were attempted for the separation of diastereomers **27a-j**.  
For the preparative thin layer chromatography: various solvent systems were tried to achieve the separation of diastereomers on preparative precoated TLC plates (Merck, Germany, 20x20 cm) which included ethyl acetate: petroleum ether (1:1, 2:1 and 3:1) and ethyl acetate: petroleum ether: acetic acid (4:5:1), however, both the diastereomers could not be resolved.  
Column chromatography: separation of mixture of diastereomers was attempted by column chromatography (silica gel, Spectrochem, 230-400 mesh) using various solvent systems ethyl acetate: petroleum ether in the ratio 1:1, 2:1, 3:1 and ethyl acetate: petroleum ether: acetic acid in the ratio 4:5:1 as well as MeOH: DCM 1:9. However, both the diastereomers could not be separated using these techniques.



## **Chapter 3**

### **Design and Synthesis of Cysteine Protease Inhibitors**

#### **Section B**

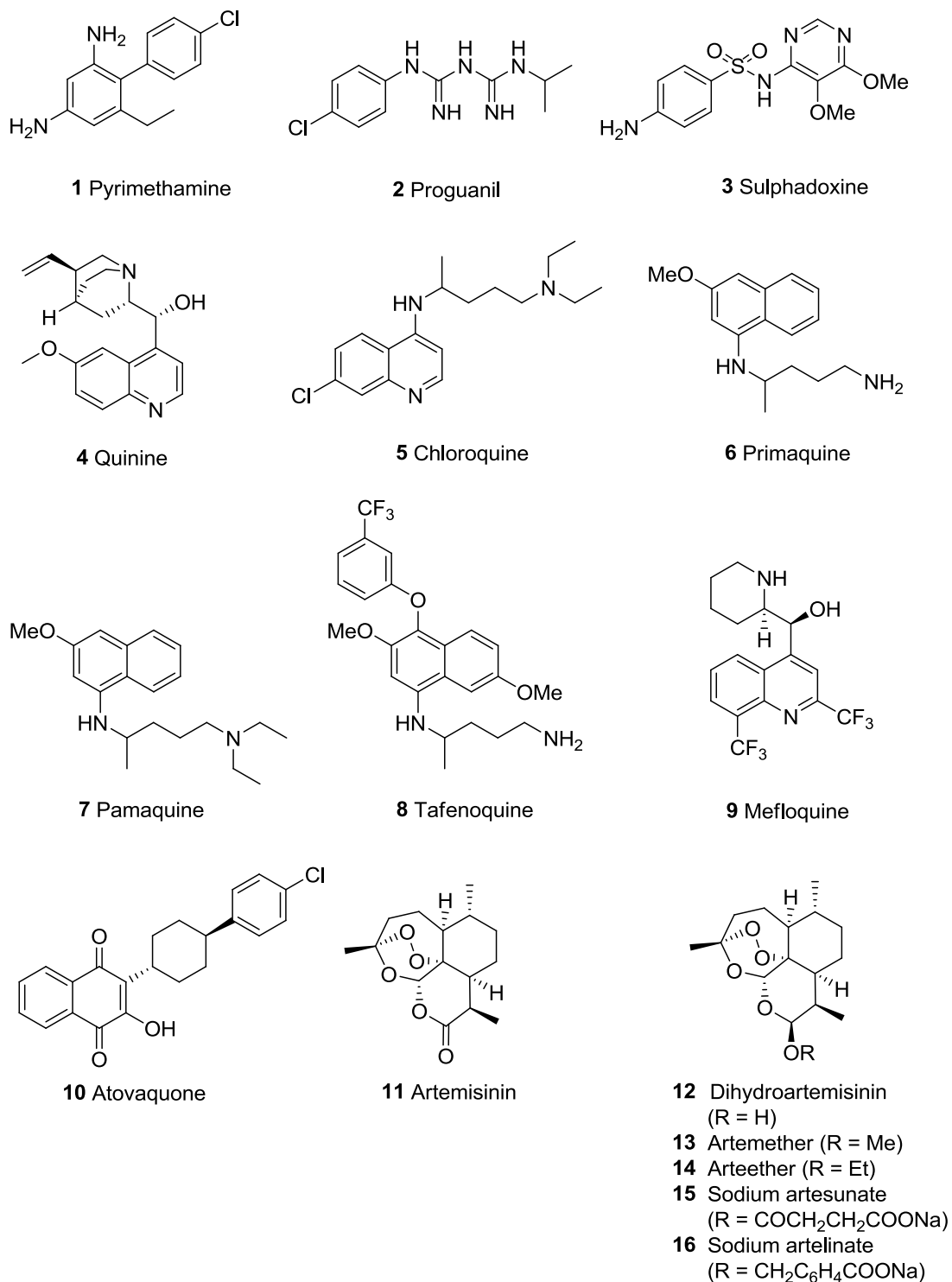
#### **Design and Synthesis of Artemisinin-Peptidyl Vinylaminophosphonate Hybrid Molecules as Falcipain-2 Protease Inhibitors**

## Introduction

Malaria continues to be a great health problem facing mankind.<sup>1</sup> Malaria is the most common of the parasitic diseases in tropical and subtropical regions and it is estimated that about 40% of the world's population lives in malaria endemic areas.<sup>2</sup> Malaria has been a major health problem in India for centuries and the details of this disease can be found even in the ancient Indian medical literature like the 'Charaka Samhita'. It is caused by protozoan parasites of the genus *Plasmodium* but in humans, it is the four species *P. falciparum*, *vivax*, *malariae* and *ovale* that are responsible for the spread of the disease. The most serious infections among these species are caused by *Plasmodium falciparum*.<sup>1-3</sup> Because of the widespread and ever increasing resistance against existing antimalarial drugs, there is increasing need for new therapeutic agents against malaria.<sup>4,1a</sup>

The currently used antimalarial drugs include: pyrimethamine, proguanil, sulfadoxine, quinine, chloroquine, primaquine, pamaquine, tefenoquine, mefloquine, atovaquone and artemisinin and its semisynthetic derivatives, such as artemether and artesunate (Figure 1).<sup>3,5</sup>

The considerable challenge in the controlling the malaria is the emergence and spread of drug resistant malaria. Chloroquine was once the most effective drug for the treatment of malaria, but resistance has been developed in the parasite against this drug.<sup>6</sup> Other antimalarial treatments exist, but they are not readily available due to their high costs or toxicity. *P. falciparum* has developed resistance to all of our available drugs; therefore it is an overwhelming cause of serious disease and death. The development of resistance to mainstay drugs like chloroquine, and controlled use of new artemisinin analogs have created an urgent need to discover new antimalarial agents.



**Figure 1.** Structures of selected anti-malarial drugs.

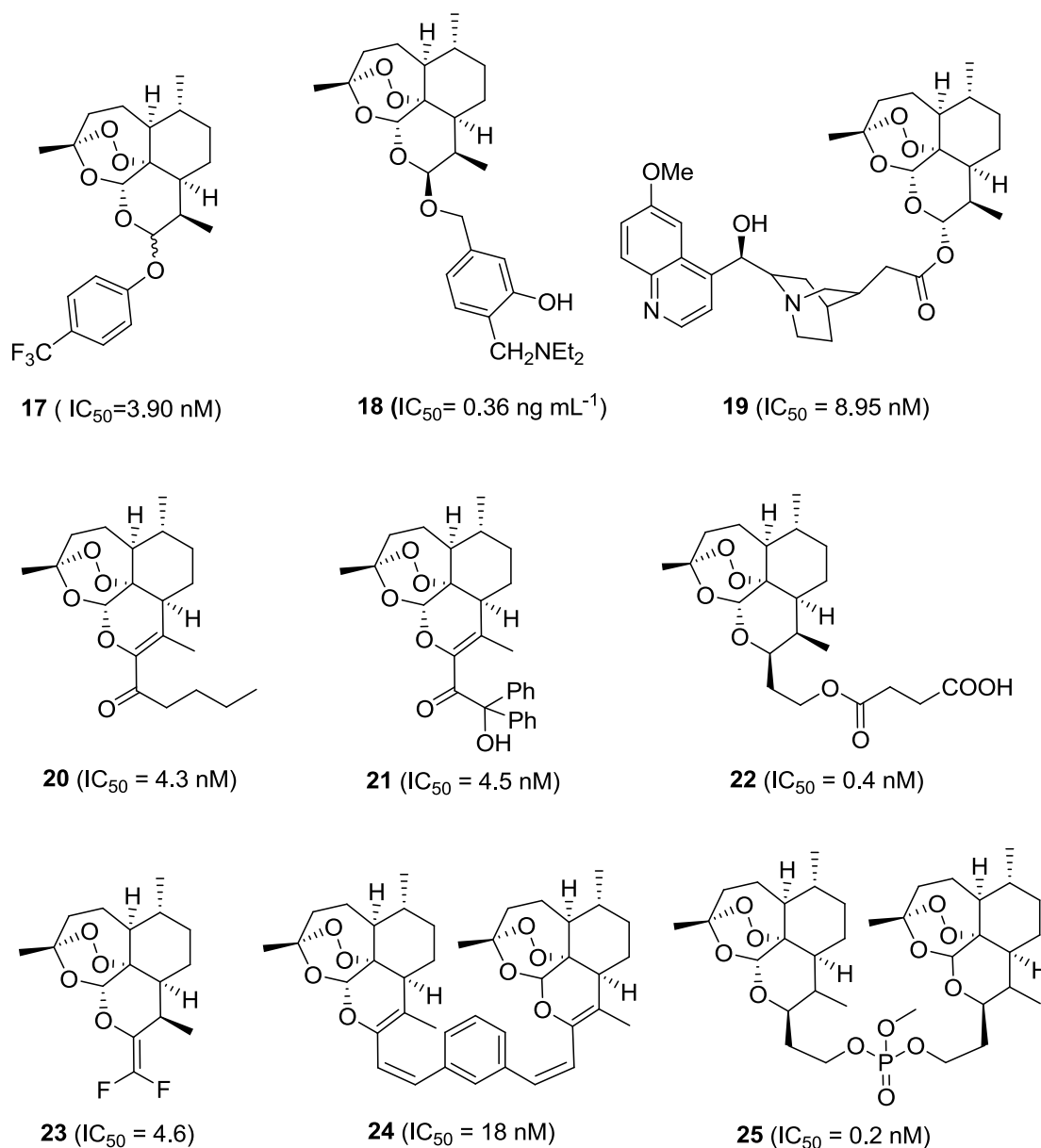
### Artemisinin

The plant *Artemisia annua* (*Asteraceae*) has been used in traditional Chinese system of medicine as a cure for fever and malaria for over 2000 years.<sup>7</sup> In the year 1972, Chinese researchers isolated the active principle artemisinin **11** (qinghaosu)



from *A. annua* and its final structure was established in 1977. It is a unique sesquiterpene lactone endoperoxide having all the five oxygen atoms on the same side of the molecule. The maximum yield of artemisinin in the plant *Artemisia annua* is 0.1%. It has superior plasmocidal and blood schizontocidal activity compared to conventional antimalarial drugs, such as chloroquine, quinine etc against malaria without any known side effects.<sup>7</sup> Artemisinin **11** is found to be active at nanomolar concentrations *in vitro* against chloroquine-sensitive as well as chloroquine-resistant strains of *P. falciparum*. However, the practical values of artemisinin is impaired by (i) its poor solubility either in oil or water,<sup>8</sup> (ii) the high rate of parasite recrudescence after treatment<sup>9</sup> and (iii) its short-plasma half life (3–5 h) and its poor oral activity.<sup>10</sup> This has prompted many researchers to design and synthesize various derivatives which can overcome some of these shortcomings. Some of these derivatives are shown in Figure 1. The carbonyl group of artemisinin **11** can easily be reduced to lactol, dihydroartemisinin **12** in high yields using sodium borohydride. The dihydroartemisinin **12** was subsequently converted to a series of semi-synthetic analogues including the oil-soluble artemether **13** and arteether **14**, and water-soluble sodium artesunate **15** and sodium artelinate **16**. These three analogs were found to be very potent antimalarial drugs effective against chloroquine-resistant strains of *P. falciparum*.<sup>7</sup> Based on structure-activity relationship (SAR), several research groups worldwide have designed and synthesized various derivatives such as hybrid molecules, dimers and fluorinated derivatives and evaluated for their plasmocidal activities (Figure 2).<sup>11</sup>

However, recently artemisinin **11** resistant strain of *P. falciparum* has been reported from the patients treated with artemisinin drug.<sup>2</sup> Also, artemisinin **11** especially dihydroartemisinin **12** has been found to be neurotoxic according to *in vivo* assays<sup>3</sup> with animal models and *in vitro* studies with neuronal cell cultures.<sup>4</sup> Therefore, there is an urgent need to develop potential antimalarial agents ideally directed against new biological targets than the prevalent drugs.



**Figure 2.** Some artemisinin derivatives and their anti-malarial activities.

### Drug Target for Malaria: Falcipain-2 and its Inhibitors

Malaria parasite ingests and degrades most of the host cell hemoglobin which is essential for the survival of the parasite itself.<sup>12</sup> Degradation of haemoglobin leads to the production of toxic heme causing membrane damage due to its peroxidative properties.<sup>13</sup> The released hemozoin is detoxified to a cyclic dimer,  $\beta$ -hemozoin and these dimers upon crystallization furnish hemozoin.<sup>14</sup> The amino acids generated after haemoglobin degradation are then incorporated into parasite proteins or utilized for

the energy metabolism.<sup>15</sup> The haemoglobin degradation by the malaria parasite is carried out by a variety of proteases present within parasite and the inhibition of this process is considered a potential drug target for the development of new antimalarial agents.<sup>16</sup> These enzymes act at a pH in the range of 4.5–5.0 *i.e.* that of the digestive food vacuole.<sup>17</sup> This biochemical process<sup>18</sup> is carried out in the food vacuole as shown in Figure 3. The aspartic proteases named plasmepsins I, II, IV and histo-aspartic protease (HAP) participate in haemoglobin degradation to peptide containing more than 20 amino acids.<sup>19</sup> This initial cleavage is considered to promote unfolding and release of the heme moiety followed by further degradation by the cysteine proteases such as falcipains-2, -2', and -3,<sup>20</sup> a metalloprotease (falcilysin)<sup>21</sup> and dipeptidyl aminopeptidase 1 (DPAP1).<sup>22</sup> to small peptides. Finally, small peptides are then pumped out of the food vacuole into the cytoplasm and an amino peptidase activity provides amino acids essential for parasites survival.<sup>23</sup> The inhibition of these proteases turns out to be lethal for parasites.

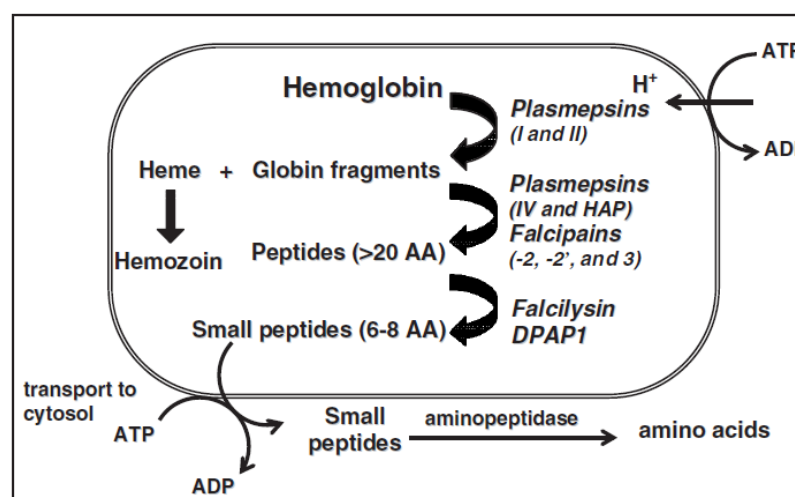


Figure 3. Hemoglobin degradation pathway in plasmodium food vacuole.<sup>12b</sup>

The cysteine proteases present in *P. falciparum*, *e.g.* falcipain-2 and falcipain-3<sup>20</sup> are considered to be the ideal drug-targets for development of new chemotherapeutic agents. Many specific inhibitors of falcipain-2 have been synthesized and evaluated as potential antimalarial agents which includes peptidyl fluromethyl ketones,<sup>24</sup> vinylsulfones,<sup>25</sup> peptidyl aldehydes,<sup>26</sup> peptidyl epoxides,<sup>27</sup> peptidomimetics based on a 1,4-benzodiazepine scaffold,<sup>28</sup> chalcones,<sup>29</sup> isoquinolines<sup>30</sup> and thiosemicarbazones<sup>31</sup> (Figure 4).

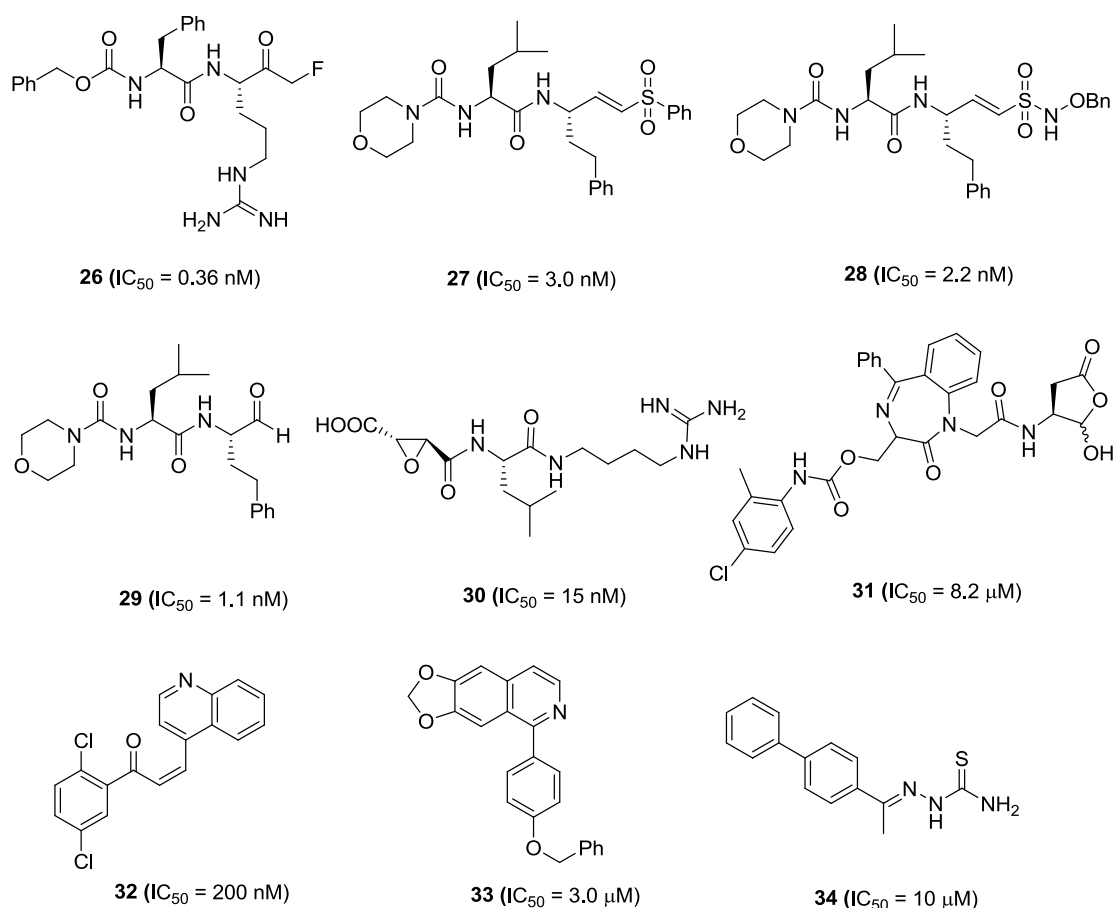


Figure 4. Some of the reported inhibitors of falcipain-2 enzyme.

### Hybrid Drugs

Hybrid drugs are considered to be formed by covalently linking two distinct chemical entities having different biological mode of action thereby creating bitherapies which would have improved biological activity and are less vulnerable to the development of drug resistance.<sup>32</sup> Recently, Walsh *et al*<sup>11d</sup> reported synthesis of a hybrid molecule **19** by combining artemisinin and quinine via ester linkage. The hybrid molecule **19** represents a unique combination of fast-acting artemisinin and slow-acting quinine into a hybrid drug for malaria for which drug resistance is a barrier to effective treatment. Since treatment with fast-acting malaria drug by itself can lead to recrudescence of the parasite *P. falciparum*. Therefore, it is believed that initially the fast acting artemisinin clears most of the parasites followed by slow acting quinine which kills the remaining parasites that haven't been killed by the fast-acting antimalarial agent and thus helps to minimize the reoccurrence of the parasite. Thus,

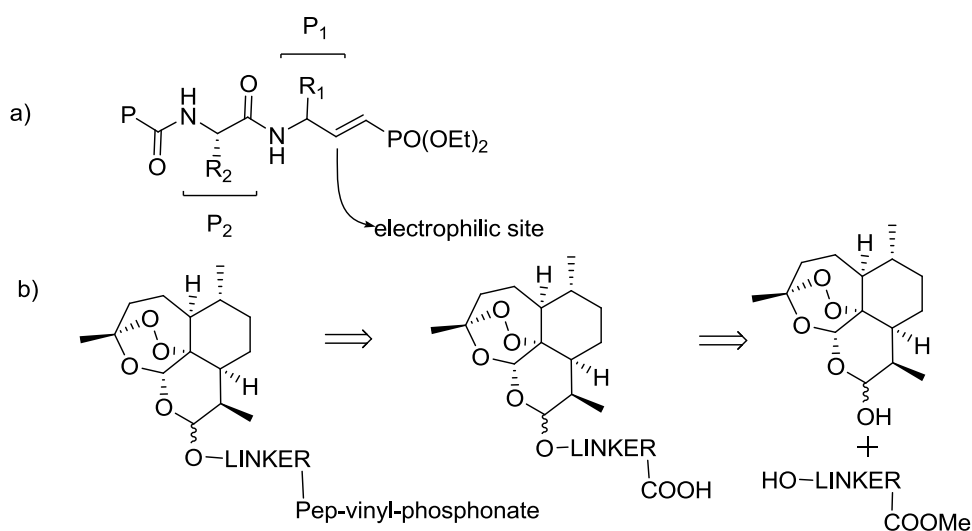
combining two suitable drug molecules could effectively minimise the limitations of either drugs and finally lead to a potent drug molecule.

## Present Work

Design and synthesis of falcipain-2 inhibitors presents excellent opportunities for developing new antimalarial drug candidates. Since falcipains belong to the cysteine class of protease and hence their inhibitors in principle would require hydrophobic interaction in  $P_1$  and  $P_2$  pockets and essentially electrophilic centre at the active-site of the enzyme.<sup>24-31</sup> We visualized that these requirements could be ideally fulfilled by our earlier designed peptidyl-vinylaminophosphonate (Figure 2a) which were found to inhibit papain, a cysteine protease (please see Section A of this chapter).

The advantage of an easily esterifiable hemiacetal functionality of dihydroartemisinin **12**, the lactol obtained by the reduction of artemisinin prompted us to design some hybrid molecules derived via hemiacetal linkage of dihydroartemisinin **12** with peptidyl-vinylaminophosphonates (Figure 2b) and study their bioefficacy against falcipain 2 protease enzyme of the malarial parasite.

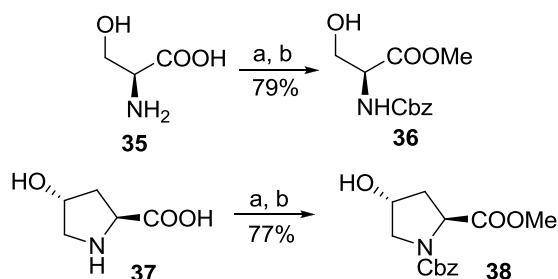
Therefore, the present work describes synthesis of artemisinin-peptidyl-vinylaminophosphonates as hybrid molecules and their efficacy against falcipain-2 protease enzyme.



**Figure 2.** (a) Possible binding required by falcipain-2; (b) artemisinin-peptidyl-vinylaminophosphonate hybrid.

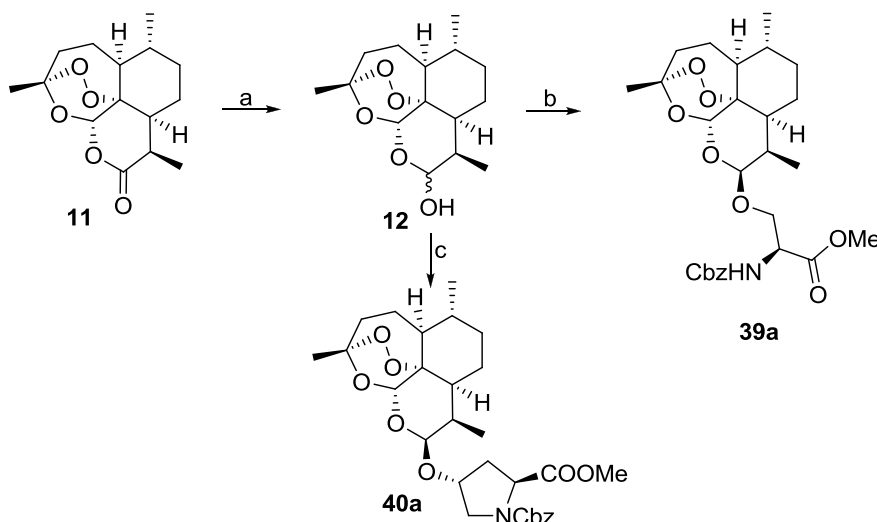
## Results and Discussion

As shown in Figure 2b, artemisinin-peptidyl-vinylaminophosphonate hybrid molecules could be synthesized by combining dihydroartemisinin **12** with peptidyl-vinylaminophosphonate coupled through suitable linkers by using etherification reaction. A linker could be any compound that has one free hydroxyl group and some functionality which could be easily converted to free acid *e. g.* methyl ester etc. Therefore, we chose amino acid based linkers, **36** and **38** fulfilling the above requirements. The linkers **36** and **38** could easily be synthesized from their corresponding free amino acids by following the literature procedure<sup>33</sup> (Scheme 1). The amine and acid functionalities of amino acids **35** and **37** were protected as benzyl carbamate and methyl ester, respectively to furnish linkers **36** and **38**.



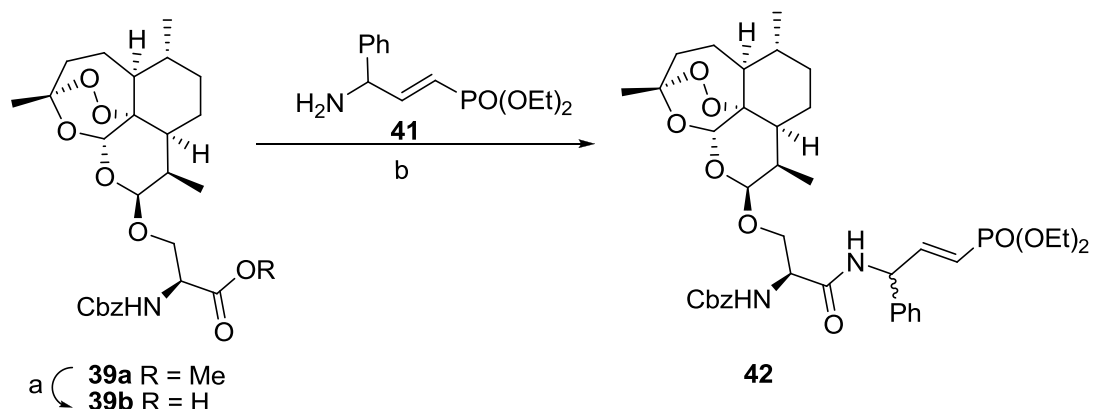
**Scheme 1.** Reagents and conditions: (a) MeCOCl, MeOH, reflux 3 h; (b) aq. NaHCO<sub>3</sub>, Cbz-Cl rt, 3 h.

The linker **36** and **38** when reacted with dihydroartemisinin **12** in the presence of BF<sub>3</sub>.Et<sub>2</sub>O as a Lewis acid, product **39a** and **40a** were obtained, respectively exclusively with β-orientation as the only major diastereomer (Scheme 2).



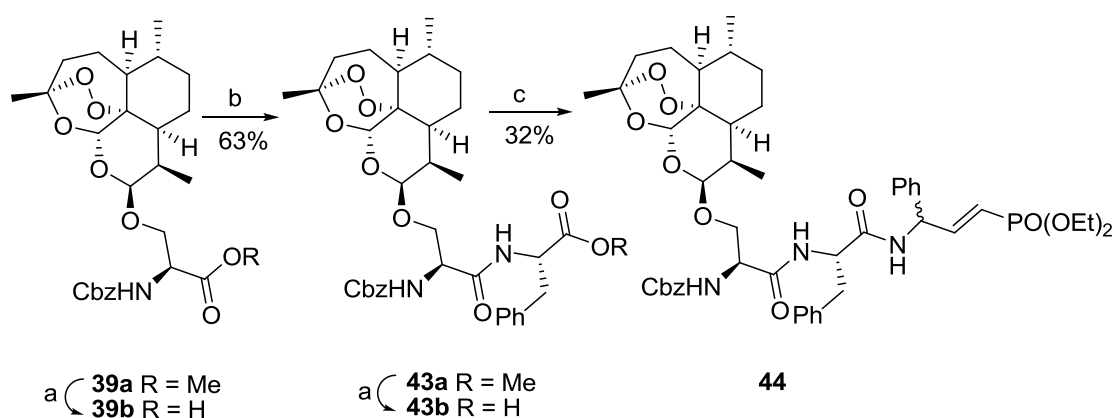
**Scheme 2.** Reagents and conditions: (a) NaBH<sub>4</sub>, MeOH, 0°C-rt, 81%; (b) **36**, BF<sub>3</sub>.Et<sub>2</sub>O, DCM, 0°C-rt, 71%; (c) **38**, BF<sub>3</sub>.Et<sub>2</sub>O, DCM, 0°C-rt, 67%.

Compound **39a** was subjected to alkaline hydrolysis (Scheme 3) and the free acid **39b** was found to be unstable, hence it was utilized as such without further purification. Freshly prepared free acid **39b** was used further for all the peptide coupling reactions. Thus, peptide coupling reaction of free acid **39b** with compound **41** (for synthesis, please see Chapter 3, Section A) yielded artemisinin-peptidyl-vinylaminophosphonate hybrid **42** as an inseparable mixture of diastereomers (1:1).

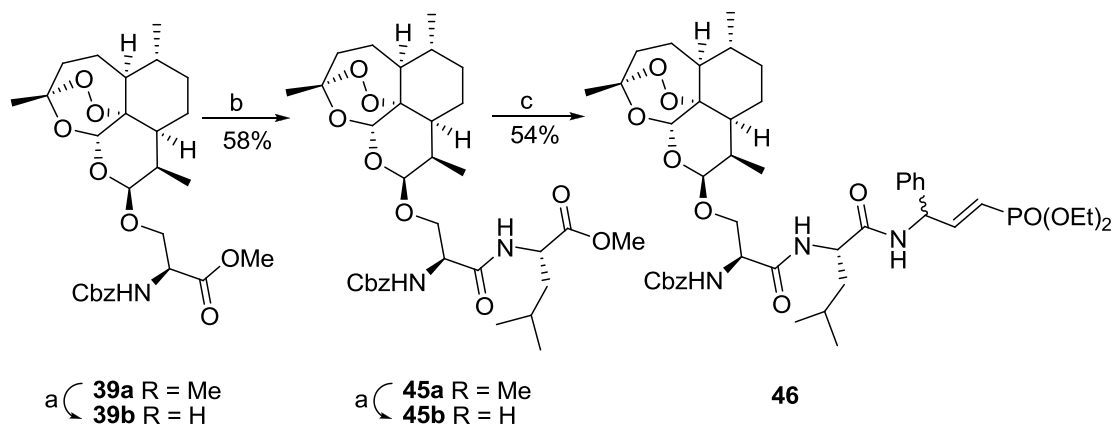


**Scheme 3.** Reagents and conditions: (a) LiOH, THF:H<sub>2</sub>O, rt, 2 h; (b) **41**, THF, DCC, HOBT, 0°C-rt, 3 h, 43%.

Similarly, compound **39b** was subjected to peptide coupling reaction with methyl ester of phenylalanine (NH<sub>2</sub>-Phe-OMe) afforded dipeptide **43a** which was again hydrolysed followed by peptide coupling reaction with **41** furnished tripeptide hybrid molecule **44** (Scheme 4). Using same synthetic strategy as outlined in Scheme 5, compounds **45a** and **46** were also synthesized.

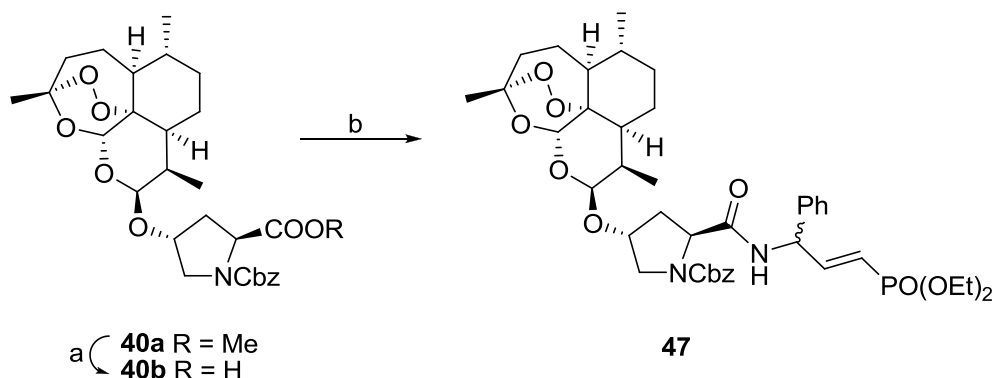


**Scheme 4.** Reagents and conditions: (a) LiOH, THF:H<sub>2</sub>O, rt, 2 h; (b) NH<sub>2</sub>-Phe-OMe, THF, DCC, HOBT, 0°C-rt, 3 h; (c) **41**, THF, DCC, HOBT, 0°C-rt, 3 h.



**Scheme 5.** Reagents and conditions: (a) LiOH, THF:H<sub>2</sub>O, rt, 2 h; (b) NH<sub>2</sub>-Leu-OMe, THF, DCC, HOBT, 0°C-rt, 3 h; (c) **41**, THF, DCC, HOBT, 0°C-rt, 3 h

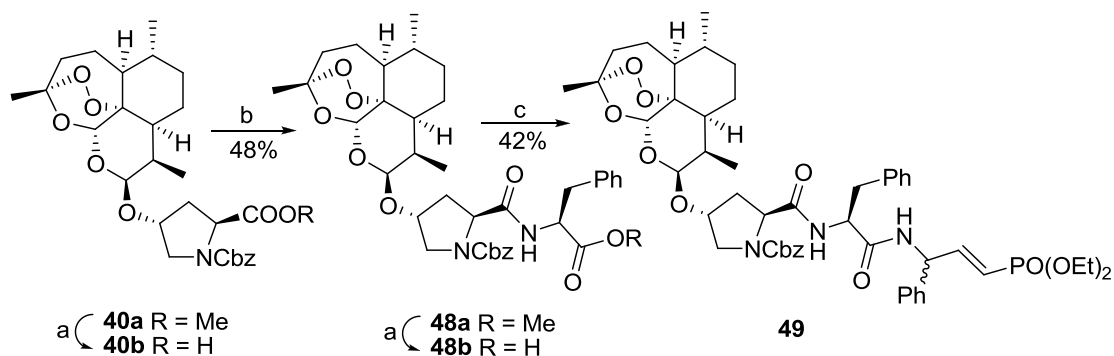
Compound **40a** on alkaline hydrolysis followed by peptide coupling reaction with **41** yielded artemisinin-peptidyl-vinylaminophosphonate hybrid **47** as an inseparable mixture of diastereomers in the ratio of 1:1.



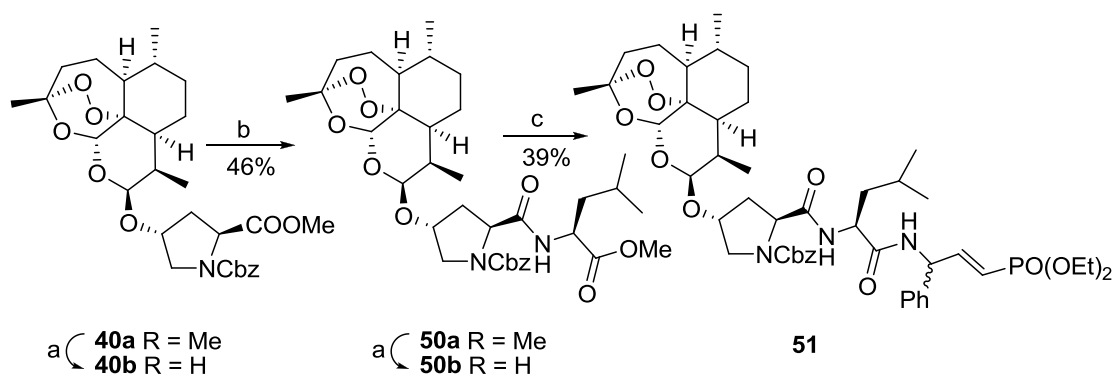
**Scheme 6.** Reagents and conditions: (a) LiOH, THF:H<sub>2</sub>O, rt, 2 h; (b) **41**, THF, DCC, HOBT, 0°C-rt, 3 h, 51%.

Alkaline hydrolysis of compound **40a** afforded the free acid **40b** which on peptide coupling reaction with methyl ester of phenylalanine (NH<sub>2</sub>-Phe-OMe) furnished dipeptide **48a** which was again hydrolysed followed by peptide coupling reaction with **41** furnished the desired compound **49** (Scheme 7). By following above mentioned set of reactions using methyl ester of leucine, synthesis of compounds **50a** and **51** were accomplished as depicted in Scheme 8.





**Scheme 7.** Reagents and conditions: (a) LiOH, THF:H<sub>2</sub>O, rt, 2 h; (b) NH<sub>2</sub>-Phe-OMe, THF, DCC, HOBT, 0°C-rt, 3 h; (c) **41**, THF, DCC, HOBT, 0°C-rt, 3 h.



**Scheme 8.** Reagents and conditions: (a) LiOH, THF:H<sub>2</sub>O, rt, 2 h; (b) NH<sub>2</sub>-Lue-OMe, THF, DCC, HOBT, 0°C-rt, 3 h; (c) **41**, THF, DCC, HOBT, 0°C-rt, 3 h.

## Biological Activities

All the synthesized artemisinin-peptidyl-vinylaminophosphonate hybrid molecules (**42**, **44**, **46**, **47**, **49** and **51**) were assayed for their inhibition activity against falcipain-2 protease enzyme. The activity results of the bioassay of all the synthesized compounds are expressed in IC<sub>50</sub> values and are summarized in Table 1.

**Table 1.** *In vitro* inhibitory effects of synthesized hybrid molecules against falcipain-2 enzyme.

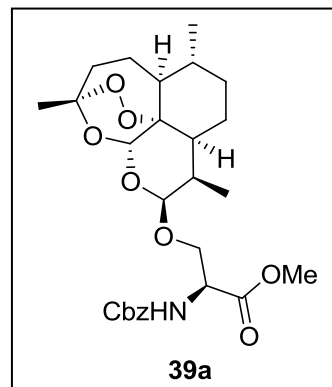
Entry	Compound	IC <sub>50</sub> values ( $\mu$ M)
1	<b>42</b>	> 100
2	<b>44</b>	3
3	<b>46</b>	4
4	<b>47</b>	> 100
5	<b>49</b>	10
6	<b>51</b>	12

The bioassay results summarized in Table 1 indicate that the artemisinin-peptidyl-vinylaminophosphonate hybrid molecules (**44**, **46**, **49**, **51**) having phenylalanine or leucine in P<sub>2</sub> pocket were more active than hybrid molecules (**42** and **47**) having serine or hydroxyproline, respectively in the P<sub>2</sub> pocket. The best inhibitor of falcipain 2 among all the synthesized hybrid molecules was found to be compound **44** bearing a phenylalanine in the P<sub>2</sub> pocket and serine in P<sub>3</sub> pocket, exhibiting IC<sub>50</sub> value of 3 μM. Compound **46** was also found to be very active against falcipain-2 enzyme with IC<sub>50</sub> value of 4 μM having leucine and serine in P<sub>2</sub> and P<sub>3</sub> pocket, respectively. On the contrary, the corresponding compounds **49** and **51** having hydroxyproline in P<sub>3</sub> pocket showed IC<sub>50</sub> 10 μM and 12 μM, respectively and were found to be 3 times less active than compounds **44** and **46** having serine in the P<sub>3</sub> pocket.

## Experimental

### 10 $\beta$ -((S)-methyl 2-(benzyloxycarbonylamino)-propanoate-3-yl-oxy)dihydroartemisinin (**39a**).

To a solution of dihydroartemisinin **12** (5.7 g, 20 mmol) and alcohol **36** (6.07 g, 24 mmol) in DCM (80 mL), BF<sub>3</sub>.Et<sub>2</sub>O (0.5 mL) was added. The resulting mixture was stirred at rt for 3 h and then washed with aqueous NaHCO<sub>3</sub> (20 mL) followed by brine (20 mL). The organic layers were dried over (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuum to afford the crude product which was purified by column chromatography (silica gel) using ethyl acetate:



petroleum ether (1:9) as eluant to furnish the pure product **39** (7.3 g, 71%) as colourless syrup;  $[\alpha]_D^{20} = +57.76$  (*c* 1.0, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>): 3439, 3019, 2955, 2876, 1723, 1698, 1505, 1455, 1377, 1215, 1028 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  0.82 (d, *J* = 7.3 Hz, 3H), 0.92 (d, *J* = 5.7 Hz, 3H), 1.13-1.33 (m, 3H), 1.41 (s, 3H), 1.46-1.74 (m, 5H), 1.81-1.89 (m, 1H), 1.91-2.07 (m, 1H), 2.28-2.43 (m, 1H), 2.57-2.65 (m, 1H), 3.74 (s, 3H), 3.87-4.10 (m, 2H), 4.52-4.60 (m, 1H), 4.74 (d, *J* = 3.5 Hz, 1H), 5.13 (s, 2H), 5.37 (s, 1H), 5.74 (d, *J* = 8.6 Hz, NH), 7.34-7.39 (s, 5H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  12.8, 20.3, 24.4, 24.6, 26.1, 30.7, 34.5, 36.4, 37.4, 44.2, 52.5, 52.5, 54.5, 67.1, 69.9, 80.9, 87.9, 103.0, 104.2, 128.1, 128.2, 128.5, 136.2, 155.9, 170.8; (ESI): *m/z* 542.8 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>27</sub>H<sub>37</sub>NO<sub>9</sub>: C, 62.41; H, 7.18; N, 2.70. Found: C, 62.53; H, 7.31; N, 2.84.

#### General procedure for hydrolysis of methyl ester:

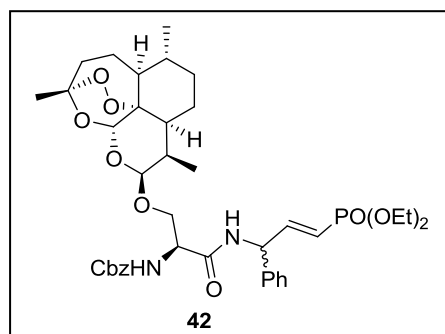
Ester (1.5 mmol) was dissolved in THF (17 mL) and a solution of aq. LiOH (2 M, 7 mL) was added to it at rt with stirring. After completion of the reaction (TLC), THF was evaporated and the remaining aqueous layer was neutralized with acetic acid. The compound was extracted with ethyl acetate (3x20 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum to obtain the crude product, which was purified by silica gel column chromatography with ethyl acetate: petroleum ether (3:2 to 4:1) as eluant.

**General procedure for peptide coupling reaction:**

Amine (0.5 mmol), HOBt (77 mg, 0.5 mmol) and protected amino acid (0.5 mmol) were dissolved in dry THF (2 mL) and the resulting solution was stirred in an ice-cooled water bath then DCC (123 mg, 0.6 mmol) was added to it. Stirring was continued for 1 h at 0°C and then an additional 1 h at rt. The solid which precipitated was removed by filtration and the solvent evaporated in vacuum. The crude peptide derivative was purified by chromatography on a silica gel column with ethyl acetate: petroleum ether (3:2 to 4:1) as eluant to furnish the corresponding peptide.

**10β(Benzyl (2S)-1-((E)-3-(diethoxyphosphoryl)-1-phenylallylamino)-1-oxopropan-2-ylcarbamate-3-yl-oxy)dihydroartemisinin (42).**

Yield: 43%; colourless syrup; IR (CHCl<sub>3</sub>): 3428, 3019, 2929, 1687, 1601, 1496, 1450, 1394, 1216, 1029 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 0.74 (d, *J* = 7.3 Hz, 3H), 0.88 (d, *J* = 7.5 Hz, 3H), 1.18-1.53 (m, 14H), 1.40 (s, 3H), 1.80-1.85 (m, 1H), 1.95-2.05 (m, 1H), 2.11-2.61 (m, 2H), 3.22-3.87 (m, 2H), 3.94-4.31 (m, 4H), 4.48 (m, 4H), 4.78 (d, *J* =

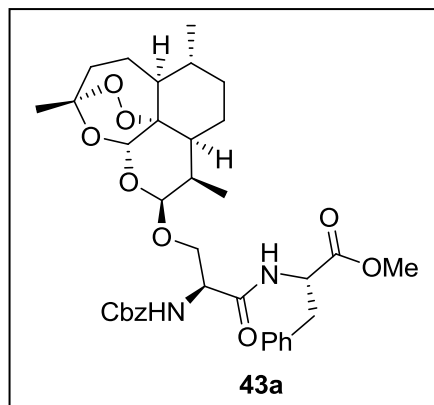


3.4 Hz, 1H), 5.11 (s, 2H), 5.35 (s, 1H), 5.70-5.91 (m, 2H), 6.74-6.97 (m, 1H), 7.19-7.59 (m, 10H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ 12.8, 16.3, 20.3, 24.4, 24.5, 26.1, 30.7, 34.5, 36.4, 37.3, 44.1, 52.4, 54.6, 55.3 (d, <sup>3</sup>*J*<sub>PC</sub> = 22.3 Hz), 61.9, 62.1, 67.2, 69.3, 80.9, 87.9, 101.8, 104.1, 117.8 (d, <sup>1</sup>*J*<sub>PC</sub> = 181.5 Hz), 127.2, 128.1, 128.2, 128.6, 128.7, 129.1, 135.9, 138.4, 150.4 (d, <sup>2</sup>*J*<sub>PC</sub> = 5.1 Hz), 156.1, 168.9; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 17.60; (ESI): *m/z* 779.6 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>39</sub>H<sub>53</sub>N<sub>2</sub>O<sub>11</sub>P: C, 61.89; H, 7.06; N, 3.70. Found: C, 61.95; H, 7.14; N, 3.78.

**10β-((S)-Methyl 2-((S)-2-(benzyloxycarbonylamino)-propanamido)-3-phenylpropanoate-3-yl-oxy)dihydroartemisinin (43a).**

Yield: 63%; colourless syrup; [α]<sub>D</sub><sup>20</sup> = +51.33 (*c* 1.0, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>): 3428, 3338, 3020, 2929, 2875, 1738, 1718, 1678, 1498, 1377, 1216, 1027 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 0.81 (d, *J* = 7.3 Hz, 3H), 0.91 (d, *J* = 5.8 Hz, 3H), 1.11-1.32 (m, 4H), 1.41 (s, 3H), 1.50-1.72 (m, 4H), 1.80-1.96 (m, 2H), 2.22-2.35 (m, 1H), 2.58-2.61 (m,

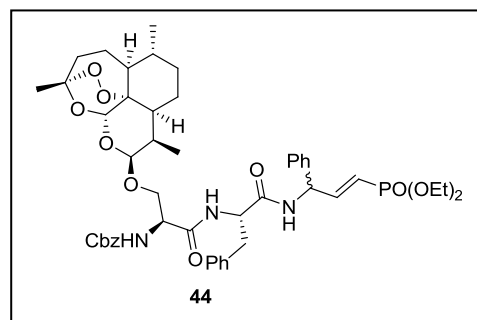
1H), 3.07-3.15 (m, 2H), 3.69 (s, 3H), 3.71-4.13 (m, 2H), 4.32-4.58 (m, 1H), 4.80 (d,  $J = 4.2$  Hz, 1H), 4.74-4.88 (m, 1H), 5.11 (s, 2H), 5.39 (s, 1H), 7.05-7.35 (m, 10H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.9, 20.4, 24.4, 24.6, 26.1, 30.7, 34.6, 36.4, 37.2, 37.9, 44.2, 52.4, 52.5, 53.4, 54.4, 67.2, 68.9, 80.9, 87.9, 102.9, 104.2, 127.2, 128.1, 128.5, 128.6, 128.6, 129.2, 135.7, 136.2, 155.9,



169.3, 171.5; (ESI):  $m/z$  689.8 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{36}\text{H}_{46}\text{N}_2\text{O}_{10}$ : C, 64.85; H, 6.95; N, 4.20. Found: C, 64.79; H, 6.90; N, 4.32.

**10 $\beta$ -((Benzyl (S)-1-((S)-1-(E)-3-(diethoxyphosphoryl)-1-phenylallylamino)-1-oxo-3-phenylpropan-2-ylamino)-1-oxopropan-2-ylcarbamate-3 oxy) dihydroartemisinin (44).**

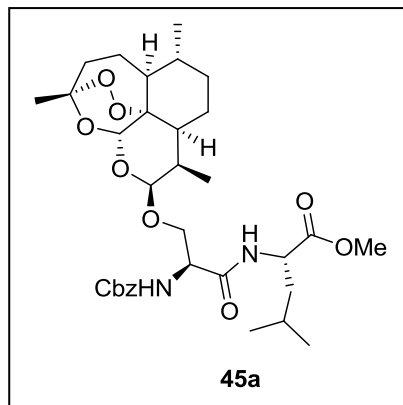
Yield: 32%; colourless syrup; IR ( $\text{CHCl}_3$ ): 3418, 3368, 3019, 2929, 1737, 1718, 1667, 1496, 1455, 1385, 1216  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.81 (d,  $J = 7.3$  Hz, 3H), 0.91 (d,  $J = 5.7$  Hz, 3H), 1.21-1.31 (m, 12H), 1.41 (s, 3H), 1.50-1.54 (m, 2H), 1.84-2.00 (m, 2H),



2.28-2.34 (m, 1H), 2.55-2.61 (m, 1H), 2.92-3.23 (m, 2H), 3.74-4.14 (m, 6H), 4.27-4.45 (m, 1H), 4.71-4.78 (m, 2H), 4.93-5.13 (m, 2H), 5.38 (s, 1H), 5.64-5.38 (m, 2H), 6.70-6.90 (m, 1H), 7.03-7.34 (m, 15H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.9, 16.3, 16.4, 20.3, 24.5, 24.6, 26.1, 30.7, 34.5, 36.3, 37.4, 37.9, 44.1, 52.4, 54.4, 55.2 (d,  $^3J_{\text{PC}} = 23.8$  Hz), 55.7, 62.1, 62.2, 67.4, 69.1, 80.9, 87.9, 103.2, 104.2, 117.5 (d,  $^1J_{\text{PC}} = 187.7$  Hz), 127.1, 127.4, 128.1, 128.2, 128.3, 128.6, 128.7, 128.9, 129.2, 135.9, 138.4, 150.4 (d,  $^2J_{\text{PC}} = 4.4$  Hz), 156.4, 169.4, 169.7;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  17.94; (ESI):  $m/z$  926.6 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{48}\text{H}_{62}\text{N}_3\text{O}_{12}\text{P}$ : C, 63.77; H, 6.91; N, 4.65. Found: C, 63.83; H, 6.86; N, 4.74.

**10 $\beta$ -((S)-Methyl 2-((S)-2-(benzyloxycarbonylamino)propanamido)-4-methylpentanoate-3-oxy)dihydroartemisinin (45a).**

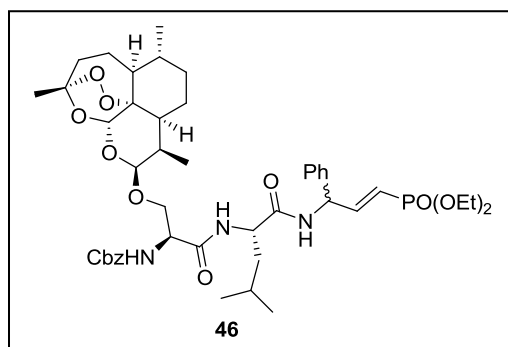
Yield: 58%; colourless syrup;  $[\alpha]_D^{20} = +75.63$  ( $c$  0.5,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ ): 3416, 3334, 3019, 2955, 2952, 1731, 1682, 1520, 1451, 1215, 1027  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.85-0.93 (m, 12H), 1.05-1.31 (m, 3H), 1.42 (s, 3H), 1.49-1.72 (m, 5H), 1.79-1.84 (m, 1H), 1.88-1.09 (m, 2H), 2.17-2.65 (m, 4H), 3.71 (s, 3H), 3.78-4.04 (m, 2H), 4.31-4.43 (m, 1H), 4.55-4.66 (m, 1H), 4.83 (d,  $J = 3.3$  Hz, 3H), 5.13 (m, 2H),



5.43 (s, 1H), 7.35 (s, 5H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.8, 20.3, 21.9, 22.7, 24.4, 24.6, 24.8, 26.0, 30.7, 33.9, 36.3, 37.2, 41.4, 44.1, 50.8, 52.1, 52.3, 52.4, 54.7, 68.5, 69.1, 80.9, 87.9, 103.0, 104.2, 128.0, 128.5, 136.3, 156.1, 169.5, 172.9; (ESI):  $m/z$  655.3 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{33}\text{H}_{48}\text{N}_2\text{O}_{10}$ : C, 62.64; H, 7.65; N, 4.43. Found: C, 62.53; H, 7.72; N, 4.57.

**10 $\beta$ -((Benzyl (S)-1-((S)-1-(E)-3-(diethoxyphosphoryl)-1-phenylallylamino)-4-methyl-1-oxopentan-2-ylamino)-1-oxopropan-2-ylcarbamate -3-oxo)dihydroartemisinin (46).**

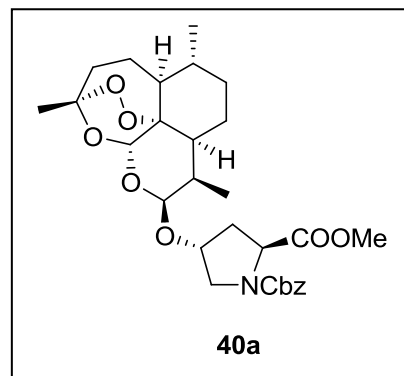
Yield: 54%; colourless syrup; IR ( $\text{CHCl}_3$ ): 3418, 3366, 3020, 2959, 1730, 1672, 1496, 1216, 1028,  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.81-0.92 (m, 12H), 1.21-1.33 (m, 9H), 1.41 (s, 3H), 1.45-1.73 (m, 7H), 1.83-2.10 (m, 3H), 2.28-2.43 (m, 1H), 2.58-2.63 (m, 1H), 3.83-4.19 (m, 2H), 3.98-4.14 (m,



4H), 4.30-4.36 (m, 1H), 4.46-4.57 (m, 1H), 4.77 (d,  $J = 3.4$  Hz, 1H), 4.97-5.12 (m, 2H), 5.40 (s, 1H), 5.73-5.92 (m, 1H), 6.77-7.04 (m, 1H), 7.23-7.34 (m, 10H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.8, 16.3, 16.4, 20.3, 21.3, 22.9, 24.5, 24.6, 24.8, 26.0, 30.7, 34.4, 36.3, 37.3, 40.5, 44.0, 51.9, 52.4, 55.1 (d,  $^3J_{\text{PC}} = 22.7$  Hz), 55.6, 62.0, 62.1, 67.4, 69.1, 80.8, 87.9, 103.3, 104.3, 117.6 (d,  $^1J_{\text{PC}} = 186.9$  Hz), 127.5, 128.1, 128.2, 128.4, 128.4, 128.6, 128.9, 135.8, 138.6, 150.6 (d,  $^2J_{\text{PC}} = 5.9$  Hz), 156.4, 169.8, 170.7;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  17.90; (ESI):  $m/z$  892.8 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{45}\text{H}_{64}\text{N}_3\text{O}_{12}\text{P}$ : C, 62.13; H, 7.41; N, 4.83. Found: C, 62.20; H, 7.50; N, 4.98.

**10 $\beta$ -((S)-1-Benzyl 2-methyl pyrrolidine-1,2-dicarboxylate-4-oxy) dihydroartemisinin (40a).**

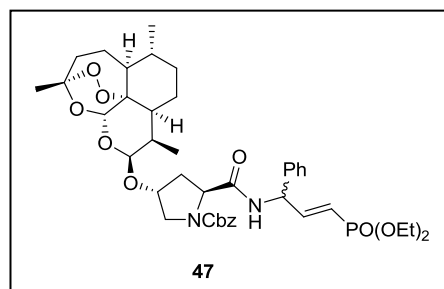
To a solution of dihydroartemisinin **12** (5.7 g, 20 mmol) and alcohol **38** (6.7 g, 24 mmol) in DCM (80 mL) was added boron trifluoride etherate (0.5 mL) at rt. The resulting mixture was stirred at room temperature for 3 h, and then washed with aqueous sodium bicarbonate (20 mL) followed by brine (20 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo to furnish the crude product. Pure compound **40**



(7.3 g, 67 %) was obtained after purification of crude reaction product by column chromatography (silica gel) using petroleum ether–ethyl acetate (9:1) as eluent. Colourless syrup;  $[\alpha]_D^{20} = +27.28$  (*c* 1.0, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>): 3436, 3019, 2956, 2876, 1747, 1704, 1605, 1455, 1422, 1358, 1215 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  0.84 (d, *J* = 7.3 Hz, 3H), 0.95 (d, *J* = 5.7 Hz, 3H), 1.21-1.39 (m, 3H), 1.43 (s, 3H), 1.56-1.71 (m, 4H), 1.85-2.23 (m, 4H), 2.29-2.43 (m, 2H), 2.58-2.62 (m, 1H), 3.52-3.60 (m, 2H), 3.76 (s, 3H), 4.38-4.54 (m, 2H), 4.79 (d, *J* = 3.5 Hz, 1H), 5.02-5.24 (m, 2H), 5.38 (s, 1H), 7.31-7.36 (m, 5H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  12.7, 20.3, 24.4, 24.6, 26.1, 30.5, 34.5, 36.4, 37.5, 37.9, 44.2, 51.5, 52.2, 52.5, 58.4, 67.2, 75.0, 80.9, 88.2, 100.7, 104.2, 127.9, 128.4, 128.5, 136.5, 155.0, 172.9; (ESI): 568.6 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>29</sub>H<sub>39</sub>NO<sub>9</sub>: C, 63.84; H, 7.20; N, 2.57. Found: C, 63.96; H, 7.31; N, 2.66.

**10 $\beta$ -((2S)-Benzyl 2-((E)-3-(diethoxyphosphoryl)-1-phenylallylcarbamoyl)pyrrolidine-1-carboxylate-4-oxy) dihydroartemisinin (47).**

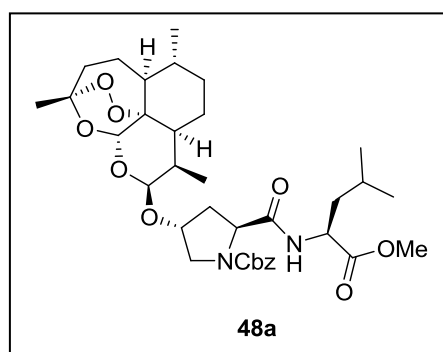
Yield: 51%; colourless syrup; IR (CHCl<sub>3</sub>): 3423, 3019, 2957, 2876, 1693, 1668, 1496, 1454, 1417, 1216, 1030 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  0.69 (d, *J* = 7.3 Hz, 3H), 0.95 (d, *J* = 5.7 Hz, 3H), 1.25-1.35 (m, 9H), 1.41 (s, 3H), 1.51-1.62 (m, 4H), 1.99-2.20 (m, 3H), 2.30-2.60 (m, 4H), 3.39-3.47



(m, 1H), 3.70-3.98 (m, 1H), 4.00-4.16 (m, 4H), 4.39-4.51 (m, 2H), 4.78 (d,  $J = 2.8$  Hz, 1H), 5.04-5.16 (m, 2H), 5.37 (s, 1H), 5.70-5.76 (m, 2H), 6.76-6.97 (m, 1H), 7.22-7.41 (m, 10H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.6, 16.3, 16.4, 20.3, 24.4, 24.6, 26.1, 30.4, 34.5, 35.5, 36.3, 37.4, 44.2, 51.7, 52.5, 55.4 (d,  $^3J_{\text{PC}} = 9.1$  Hz), 59.6, 61.9, 62.0, 67.7, 74.5, 80.9, 88.1, 99.1, 104.2, 117.2 (d,  $^1J_{\text{PC}} = 185.5$  Hz), 127.3, 127.4, 128.1, 128.3, 128.6, 129.0, 136.0, 138.5, 150.5 (d,  $^2J_{\text{PC}} = 5.12$  Hz), 156.7, 170.3;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  18.10; (ESI):  $m/z$  805.5 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{41}\text{H}_{55}\text{N}_2\text{O}_{11}\text{P}$ : C, 62.90; H, 7.08; N, 3.58. Found: C, 62.81; H, 7.18; N, 3.67.

**10 $\beta$ -((S)-Benzyl 2-((S)-1-methoxy-4-methyl-1-oxopentan-2-ylcarbamoyl)pyrrolidine-1-carboxylate-4-oxy) dihydroartemisinin (48a).**

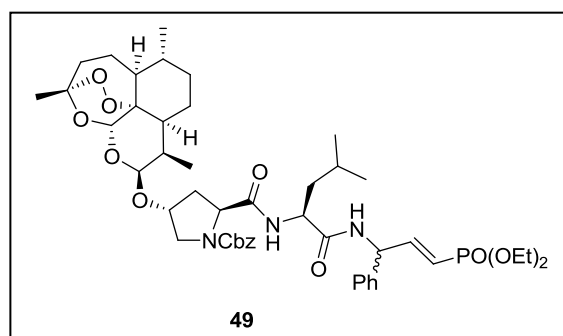
Yield: 48%; colourless syrup;  $[\alpha]_{\text{D}}^{20} = +12.0$  ( $c$  1.0,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ ): 3420, 3322, 3018, 2956, 2874, 1738, 1694, 1668, 1538, 1416, 1215, 1030  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.72 (d,  $J = 7.3$  Hz, 3H), 0.89-0.97 (m, 9H), 1.13-1.26 (m, 3H), 1.42 (s, 3H), 1.54-1.65 (m, 7H), 1.86-2.04 (m, 4H), 2.35-2.59 (m, 3H), 3.39-3.73 (m, 2H), 3.73 (s, 3H),



4.41-4.54 (m, 3H), 4.80 (d,  $J = 3.5$  Hz, 1H), 5.14 (s, 2H), 5.38 (s, 1H), 7.30-7.33 (m, 5H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.6, 20.3, 21.9, 22.7, 24.4, 24.6, 24.8, 26.1, 30.4, 33.9, 34.5, 36.3, 37.5, 41.4, 44.2, 50.9, 51.6, 52.3, 52.5, 59.4, 67.5, 74.5, 80.9, 88.1, 100.0, 104.2, 127.6, 127.9, 128.5, 136.2, 156.4, 170.9, 173.2; (ESI): 681.9 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{35}\text{H}_{50}\text{N}_2\text{O}_{10}$ : C, 63.81; H, 7.65; N, 4.25. Found: C, 63.73; H, 7.81; N, 4.34.

**10 $\beta$ -((S)-Benzyl 2-((S)-1-(E)-3-(diethoxyphosphoryl)-1-phenylallylcarbamoyl)-4-methyl-1-oxopentan-2-ylcarbamoyl)pyrrolidine-1-carboxylate-4-oxy) dihydroartemisinin (49).**

Yield: 42%; colourless syrup; IR ( $\text{CHCl}_3$ ): 3422, 3334, 3019, 2959, 2874, 1713, 1680, 1667, 1520, 1452, 1416, 1216, 1030  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.67 (d,  $J = 7.3$  Hz, 3H), 0.87-0.97 (m, 9H), 1.23-1.29 (m, 11H), 1.42

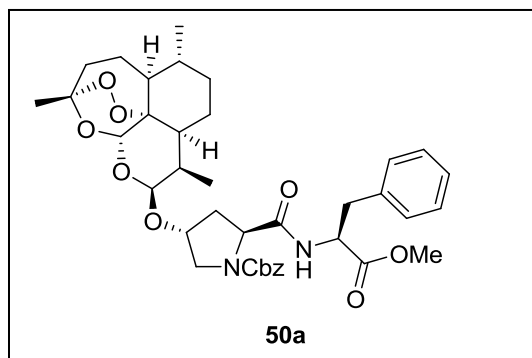




(s, 3H), 1.52-1.64 (m, 6H), 1.73-1.90 (m, 2H), 2.03-2.06 (m, 2H), 2.28-2.62 (m, 2H), 3.38-3.77 (m, 2H), 3.98-4.05 (m, 4H), 4.36-4.57 (m, 3H), 4.70 (d,  $J = 3.5$  Hz, 3H), 4.84-5.19 (m, 2H), 5.37 (s, 1H), 5.76-6.01 (m, 2H), 6.63-7.03 (m, 1H), 7.31-7.32 (m, 10H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.6, 16.2, 16.4, 20.3, 21.6, 23.0, 24.6, 24.4, 24.8, 26.1, 30.4, 34.4, 36.3, 36.8, 37.4, 40.2, 44.1, 51.6, 51.9, 52.4, 55.2, (d,  $^3J_{\text{PC}} = 22.7$  Hz), 60.4, 61.9, 62.0, 67.7, 74.2, 80.9, 88.1, 99.9, 104.2, 117.3 (d,  $^1J_{\text{PC}} = 187.7$  Hz), 127.4, 127.8, 128.0, 128.5, 128.7, 128.8, 135.8, 138.7, 150.7 (d,  $^2J_{\text{PC}} = 5.1$  Hz), 156.3, 171.2, 171.3;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  18.06; (ESI):  $m/z$  919.2 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{47}\text{H}_{66}\text{N}_3\text{O}_{12}\text{P}$ : C, 63.00; H, 7.42; N, 4.69. Found: C, 63.16; H, 7.50; N, 4.61.

**10 $\beta$ -((S)-Benzyl 2-((S)-1-methoxy-1-oxo-3-phenylpropan-2-ylcarbamoyl)pyrrolidine-1-carboxylate-4-oxy) dihydroartemisinin (50a).**

Yield: 46%; colourless syrup;  $[\alpha]_{\text{D}}^{20} = +30.8$  ( $c$  0.5,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ ): 3421, 3341, 3031, 2953, 2875, 1743, 1694, 1668, 1520, 1454, 1356, 1211  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.70 (d,  $J = 7.3$  Hz, 3H), 0.94 (d,  $J = 5.4$  Hz, 3H), 1.12-1.28 (m, 3H), 1.41 (s, 3H), 1.57-1.62 (m, 4H), 1.85-

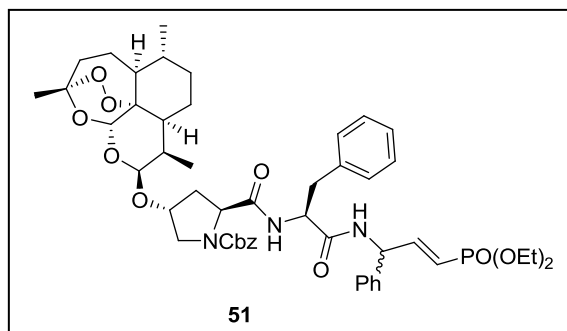


1.96 (m, 3H), 2.20-2.34 (m, 3H), 2.53-2.61 (m, 1H), 2.98-3.29 (m, 2H), 3.52-3.85 (m, 2H), 3.71 (s, 3H), 4.39-4.41 (m, 2H), 4.76-4.86 (m, 2H), 5.12 (s, 2H), 5.35 (s, 1H), 7.09-7.34 (m, 10H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.6, 20.3, 24.4, 24.6, 26.1, 30.4, 34.5, 35.5, 36.3, 37.4, 37.8, 44.2, 51.5, 52.3, 52.4, 53.3, 59.3, 67.5, 74.4, 80.9, 88.0, 100.0, 104.2, 127.0, 128.0, 128.1, 128.4, 128.5, 129.3, 135.8, 136.2, 156.3, 170.8, 171.7; (ESI): 715.5 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{38}\text{H}_{48}\text{N}_2\text{O}_{10}$ : C, 65.88; H, 6.98; N, 4.04. Found: C, 65.78; H, 7.10; N, 4.16.

**10 $\beta$ -((S)-Benzyl 2-((S)-1-(E)-3-(diethoxyphosphoryl)-1-phenylallylcarbamoyl)-1-oxo-3-phenylpropan-2-ylcarbamoyl)pyrrolidine-1-carboxylate-4-oxy) dihydroartemisinin (51).**

Yield: 39%; colourless syrup; IR ( $\text{CHCl}_3$ ): 3409, 3368, 3018, 2957, 2876, 1682, 1497, 1455, 1416, 1358, 1216, 1030  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.63 (d,  $J =$

7.2 Hz, 3H), 0.94 (d,  $J = 5.7$  Hz, 3H), 1.22-1.32 (m, 10H), 1.42 (s, 3H), 1.49-1.70 (m, 3H), 1.85-1.99 (m, 3H), 2.27-2.57 (m, 3H), 3.13-3.33 (m, 2H), 3.57-4.13 (m, 2H), 3.96-4.13 (m, 2H), 4.27-4.39 (m, 2H), 4.73-4.90 (m, 2H), 4.80-5.30 (m, 2H), 5.71-5.93 (m, 2H), 6.75-

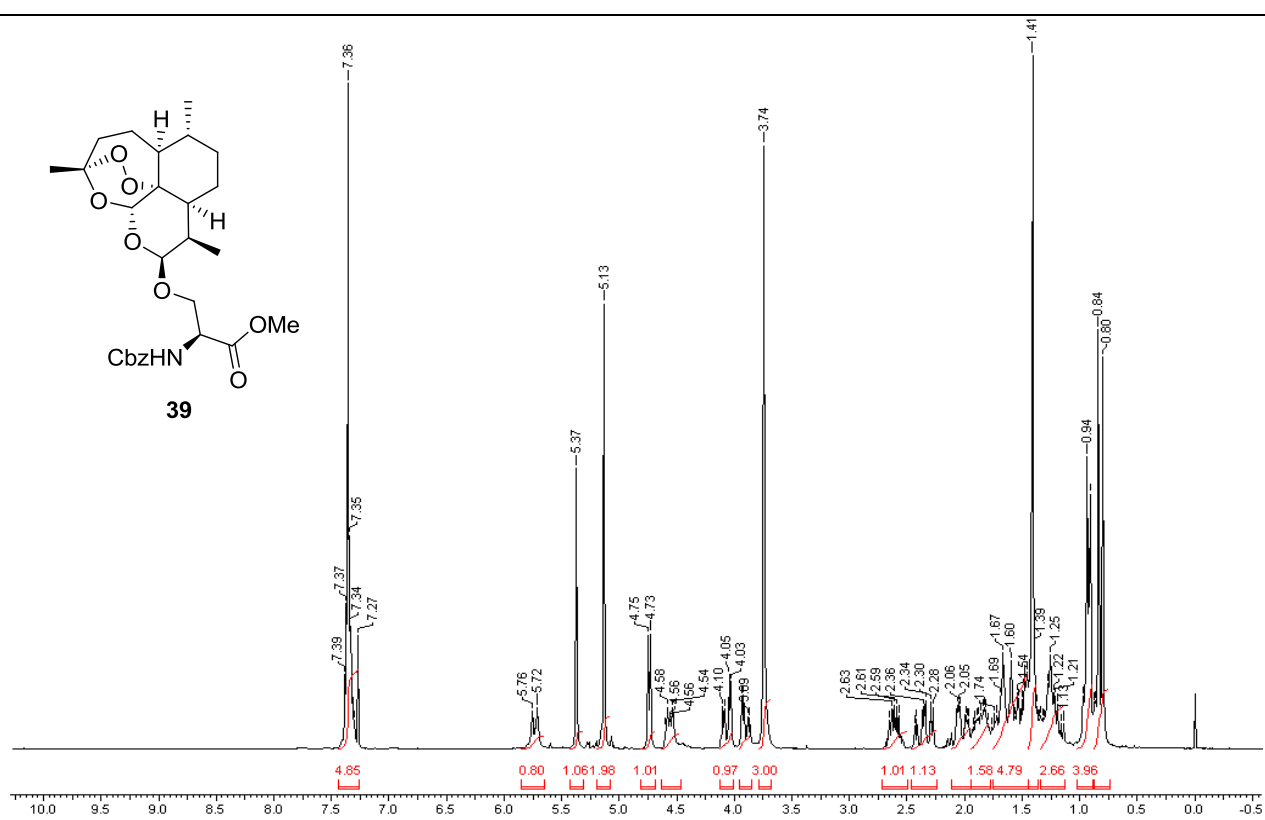
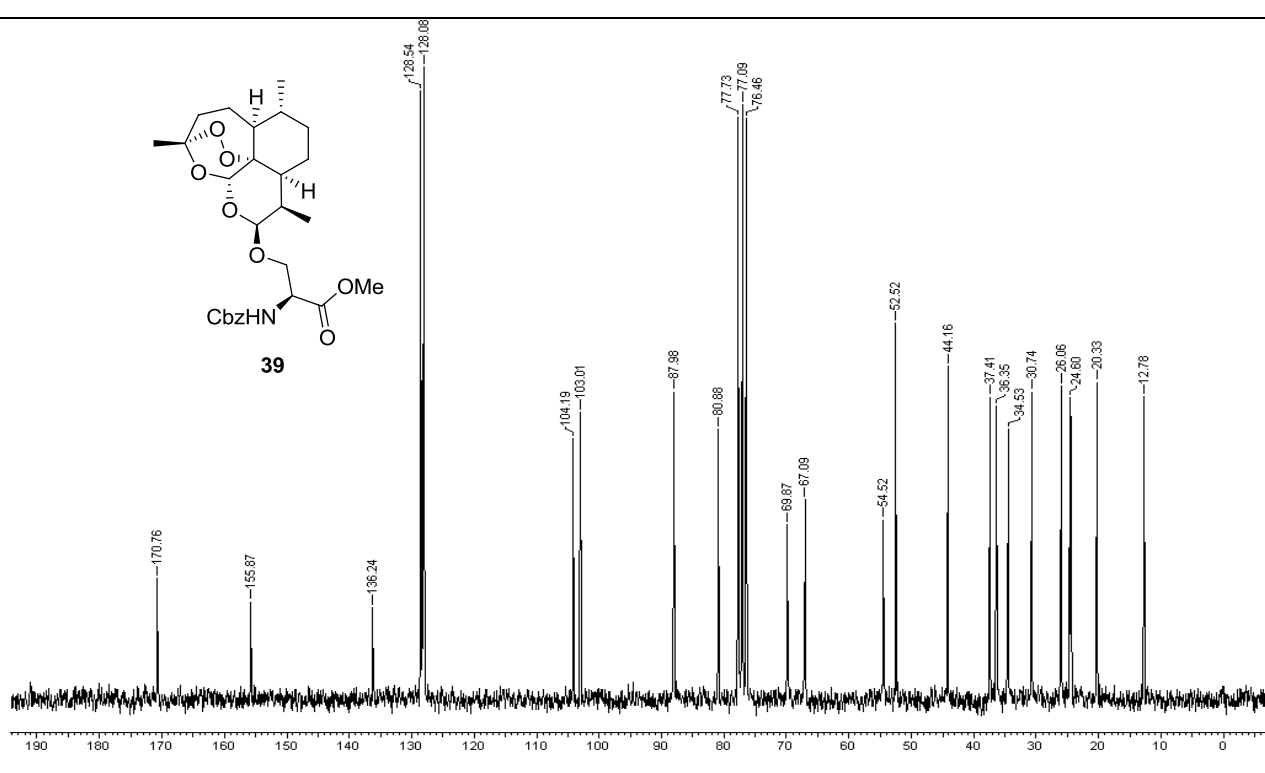


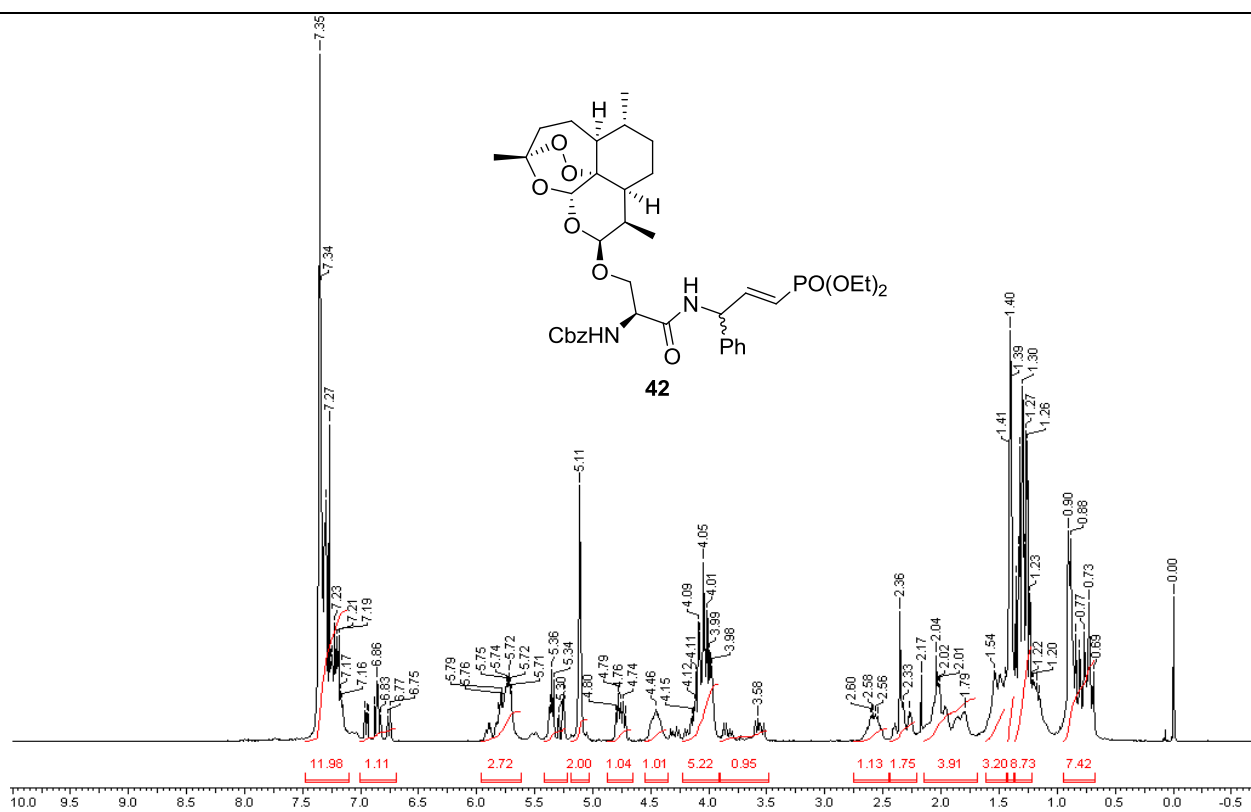
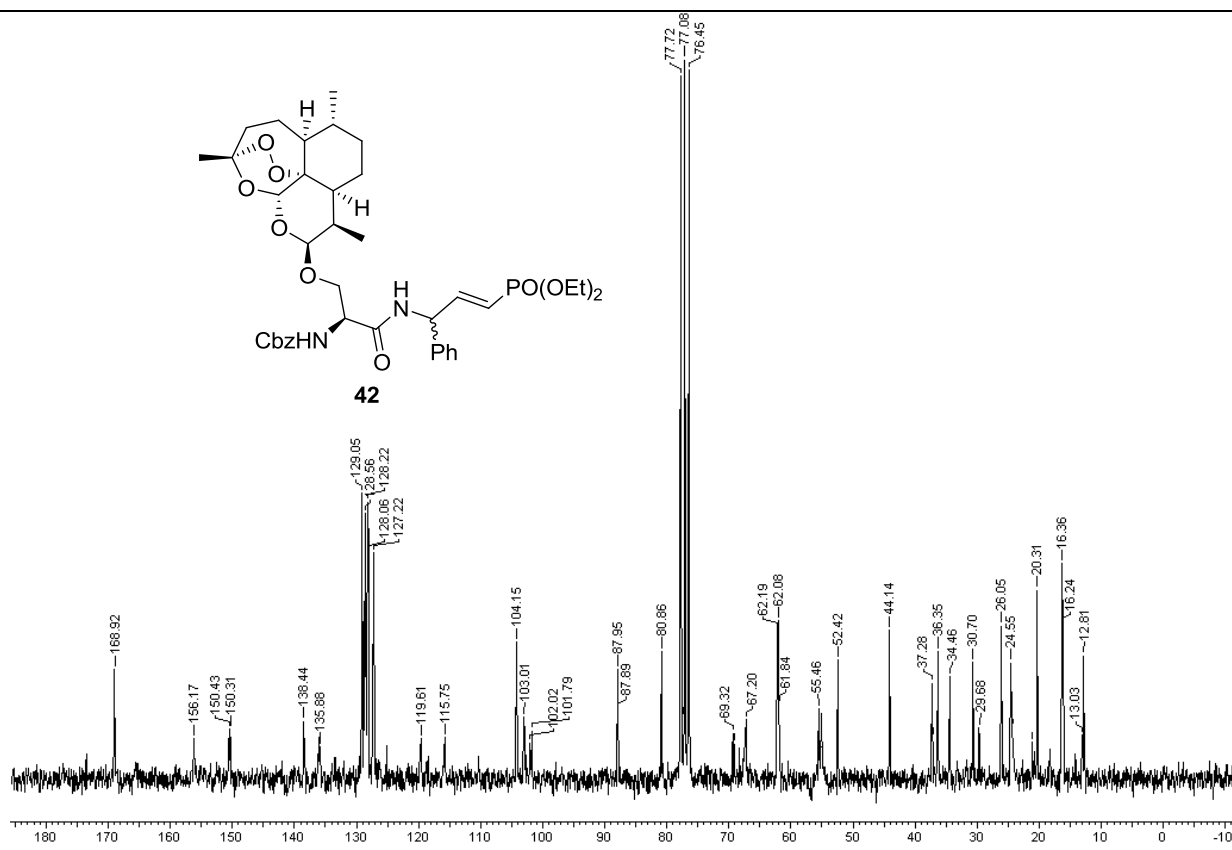
6.94 (m, 1H), 7.17-7.30 (m, 15H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.6, 16.2, 16.3, 20.3, 24.4, 24.6, 26.1, 30.4, 34.4, 36.3, 36.8, 36.9, 37.4, 44.1, 51.8, 52.4, 53.8, 55.3 (d,  $^3J_{\text{PC}} = 23.3$  Hz), 60.6, 62.1, 67.7, 73.9, 80.9, 88.1, 99.9, 104.2, 117.1 (d,  $^3J_{\text{PC}} = 187.7$  Hz), 126.9, 127.4, 127.9, 128.0, 128.5, 128.6, 128.7, 128.8, 129.2, 136.1, 136.8, 138.4, 151.5 (d,  $^2J_{\text{PC}} = 6.6$  Hz), 156.2, 169.9, 171.4;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  18.21; (ESI):  $m/z$  953.2 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{50}\text{H}_{64}\text{N}_3\text{O}_{12}\text{P}$ : C, 64.57; H, 6.94; N, 4.52. Found: C, 64.68; H, 6.84; N, 4.46.

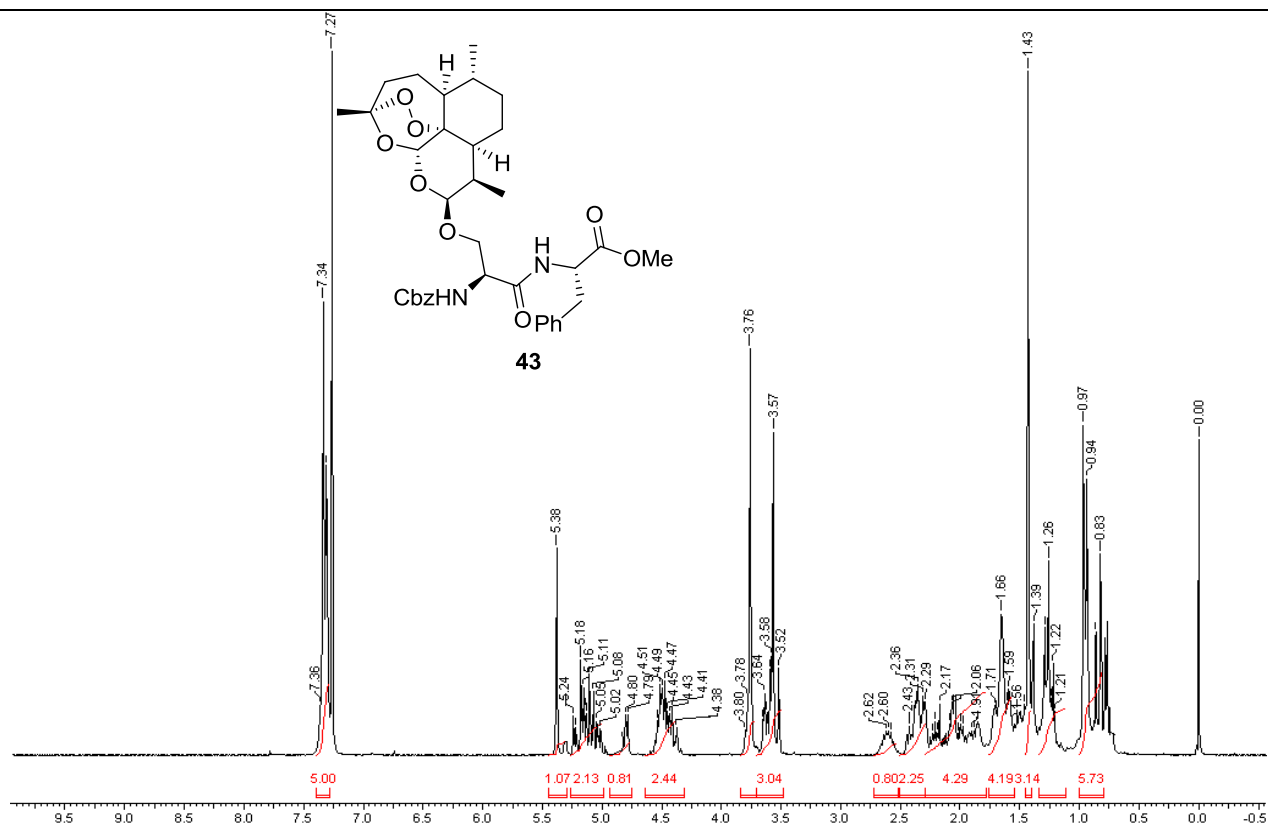
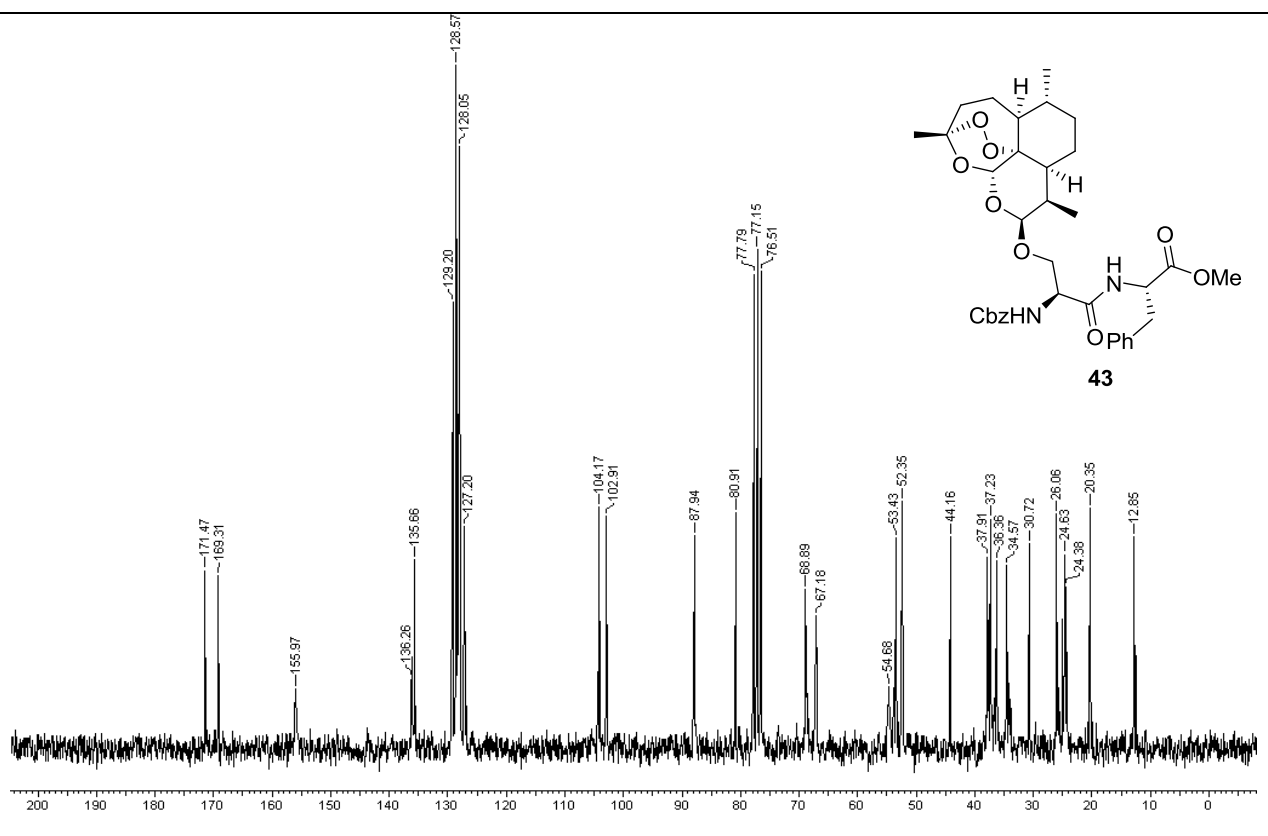
#### Bioassays of synthesized compounds against Falcipain 2 enzyme:

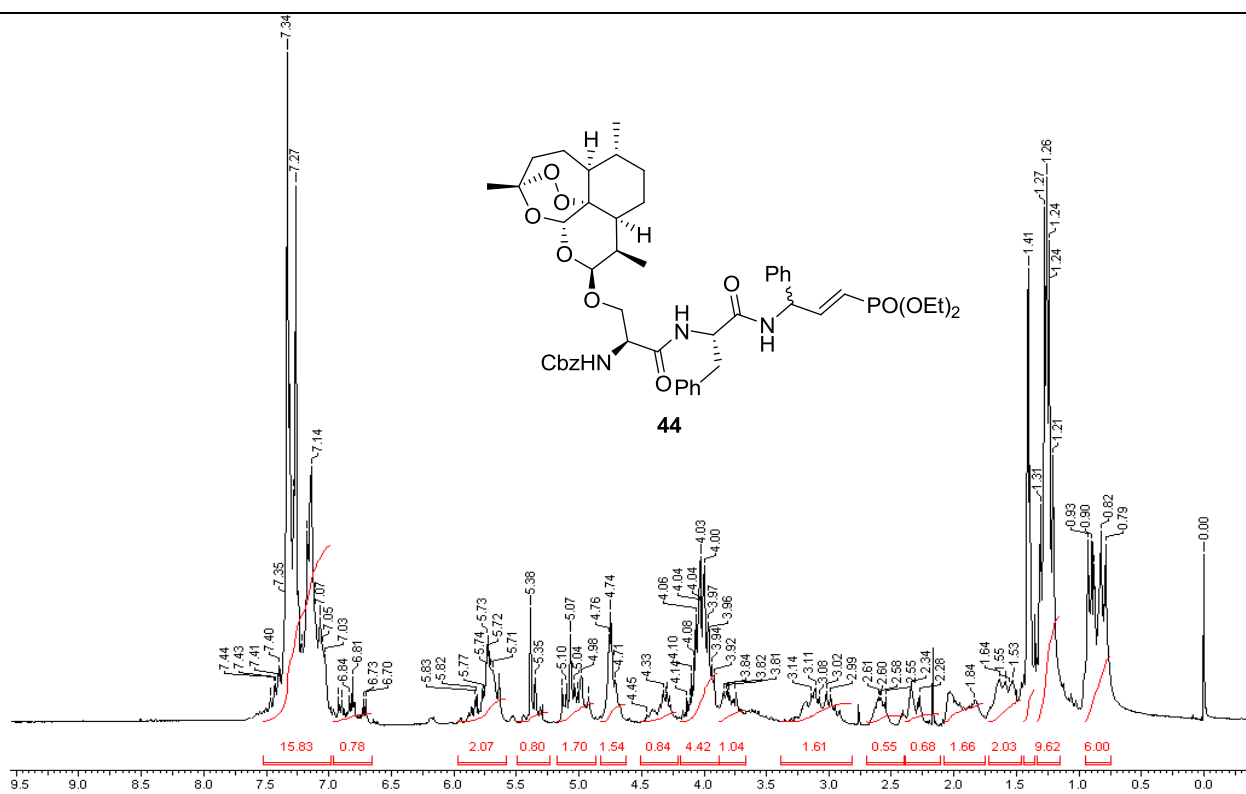
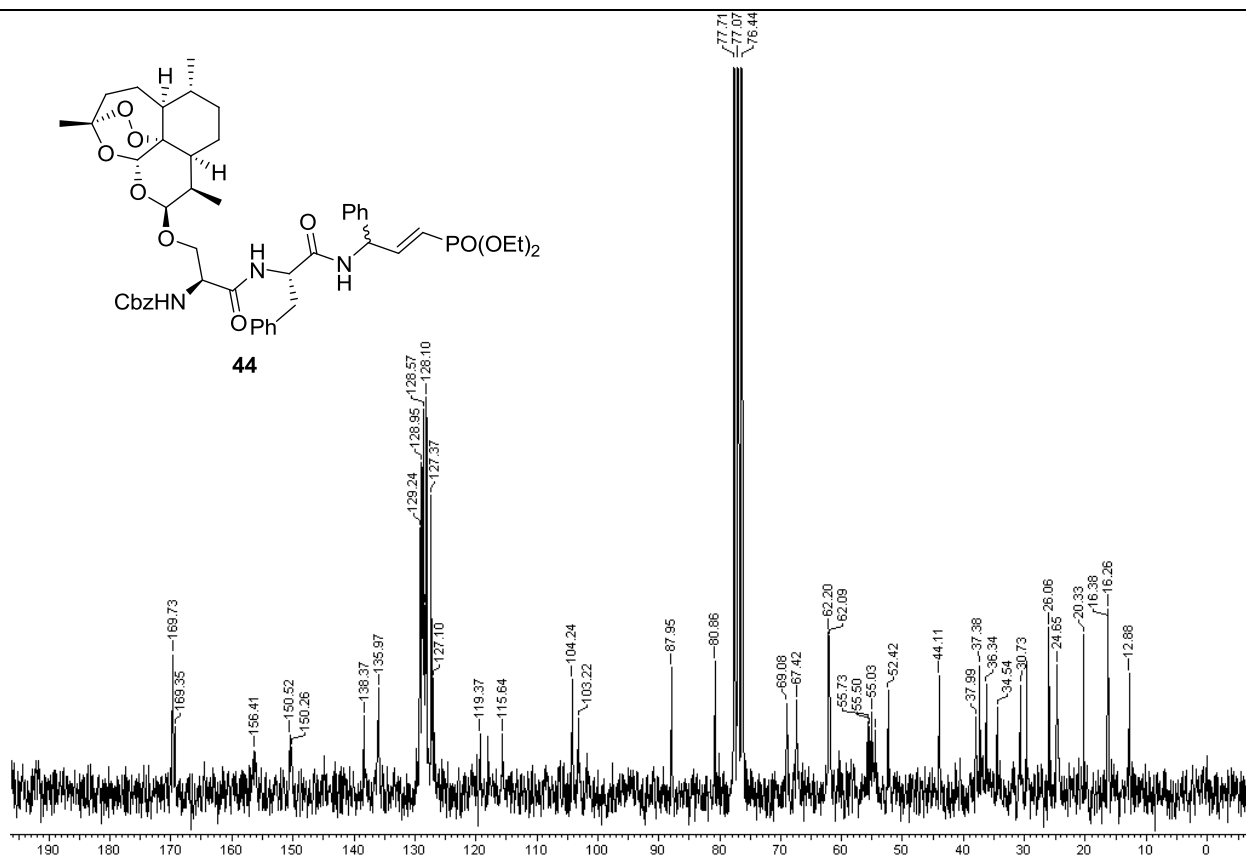
Fluorimetric assay for falcipain-2 activity was carried out following a literature procedure reported by Korde et al.<sup>34</sup> Briefly, in 3 mL of assay buffer (100 mM sodium acetate, pH 5.5, 10 mM DTT) containing 200 nM of enzyme, fluoregenic substrate Z-F-R-AMC was added at 7  $\mu\text{M}$  concentration and the release of 7-amino-4-methyl coumarin (AMC) was monitored (excitation 355 nm; emission 460 nm) over 30 min at rt using a LS50B Perkin-Elmer fluorimeter. To analyze the effect of artemisinin-peptidyl-vinylaminophosphonate hybrid molecules (**42**, **44**, **46**, **47**, **49** and **51**) on enzyme activity, recombinant falcipain-2 was preincubated with each peptide for 10 min at room temperature.  $\text{IC}_{50}$  values were determined from plots of activity against enzyme concentration.

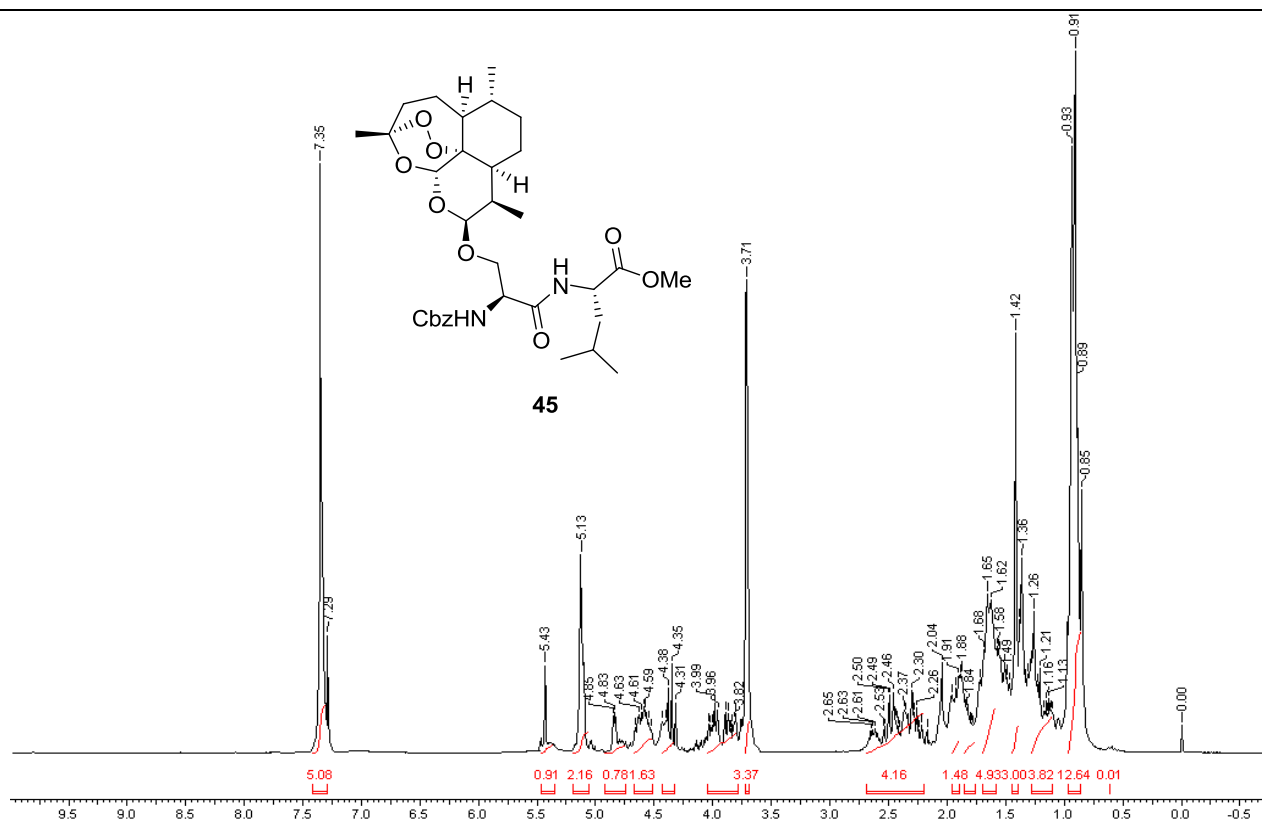
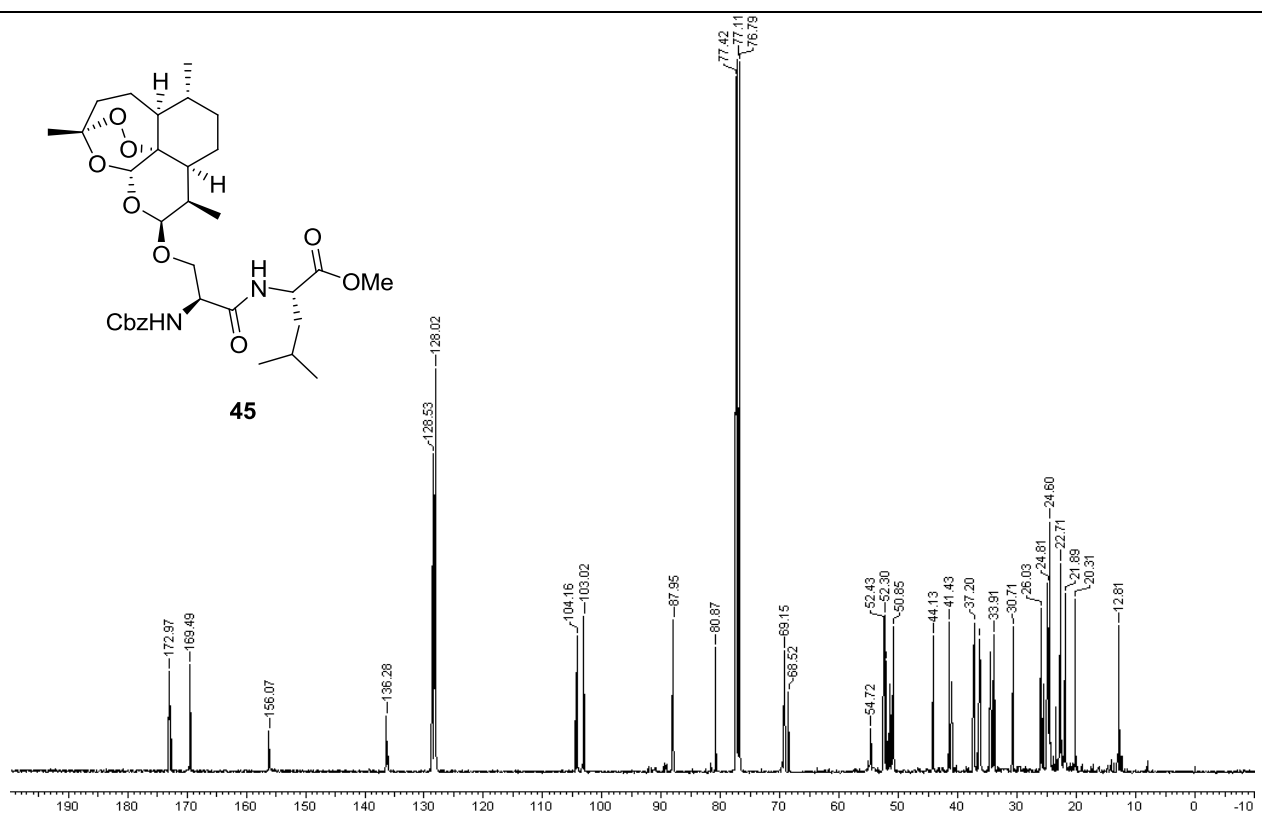
## Spectra

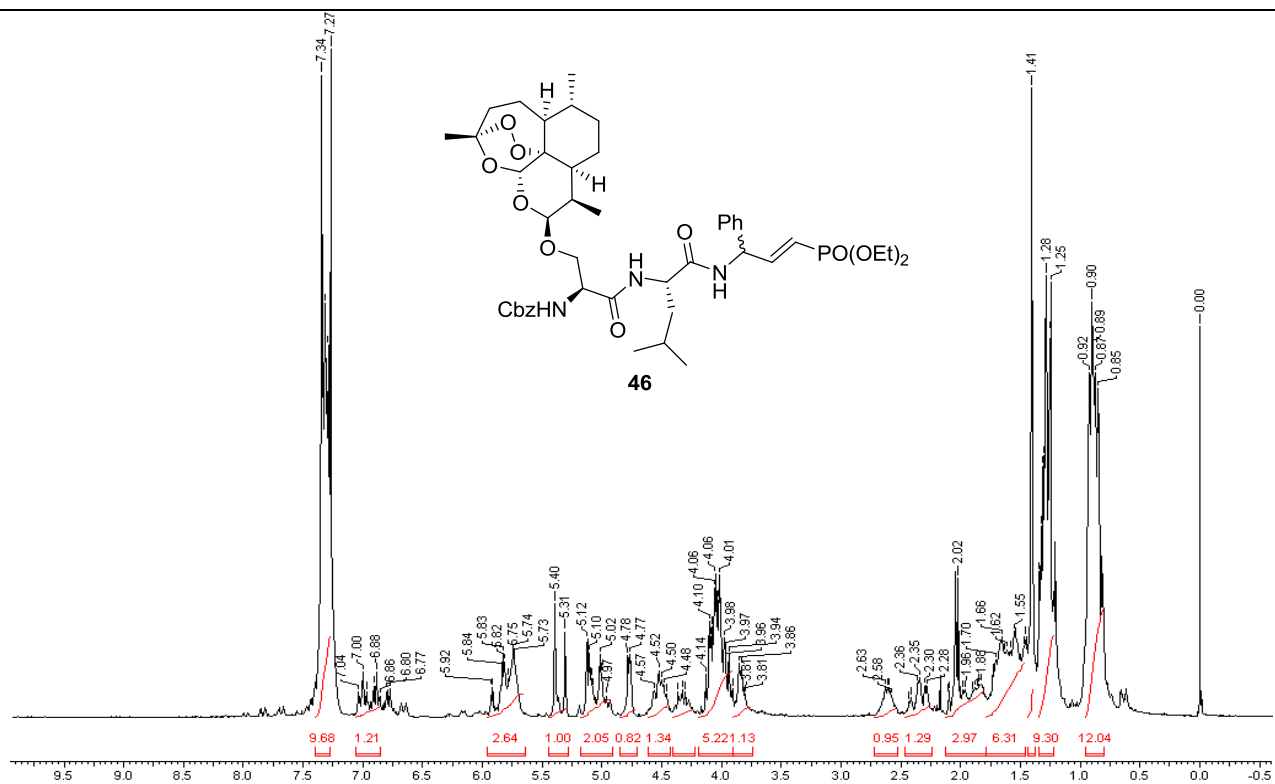
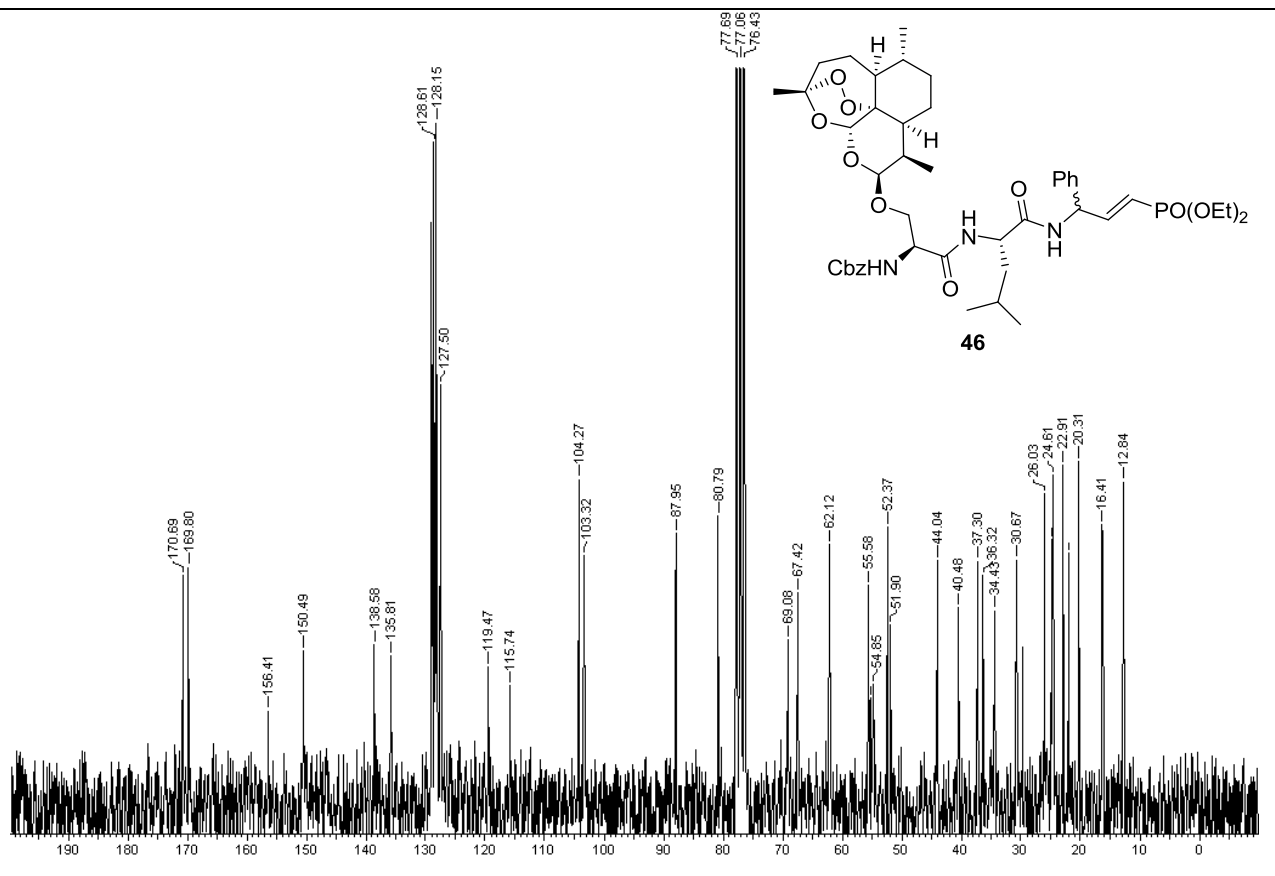
 $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **39** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **39**

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **42** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **42**

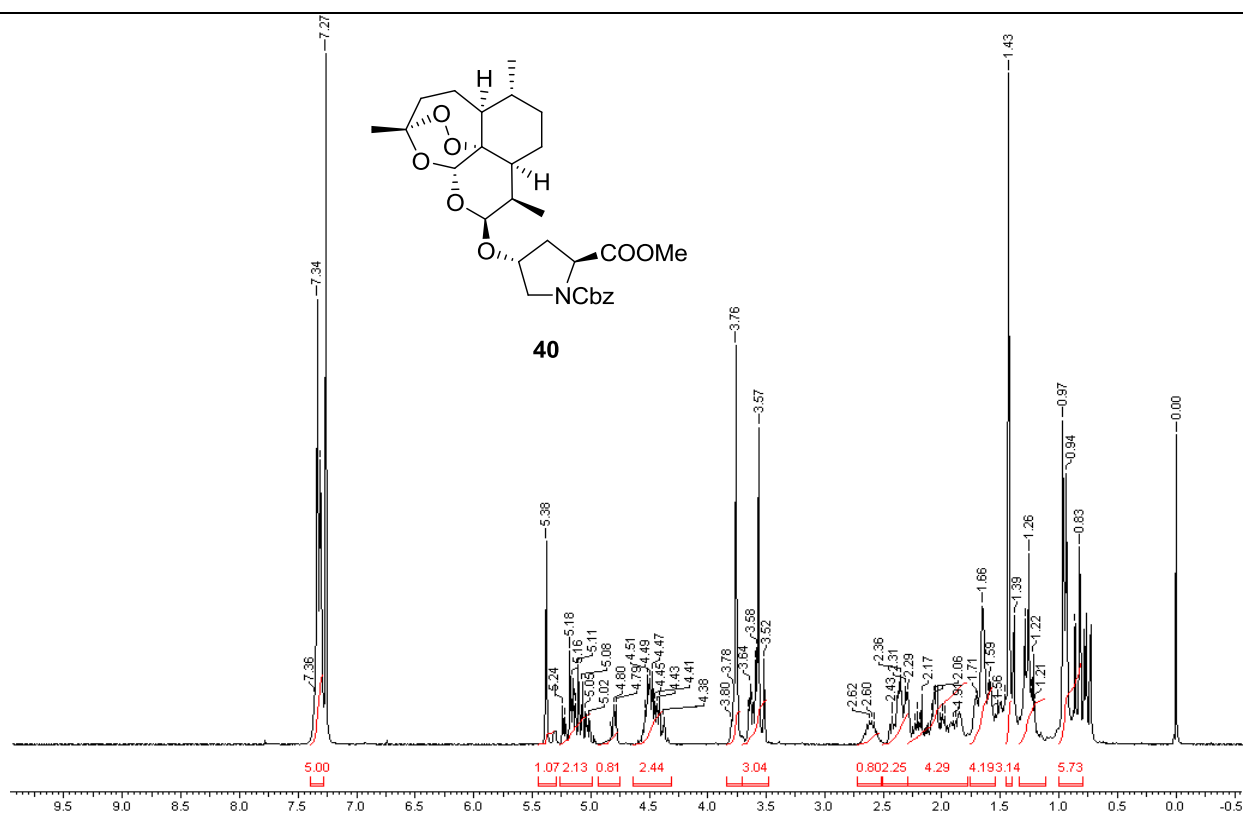
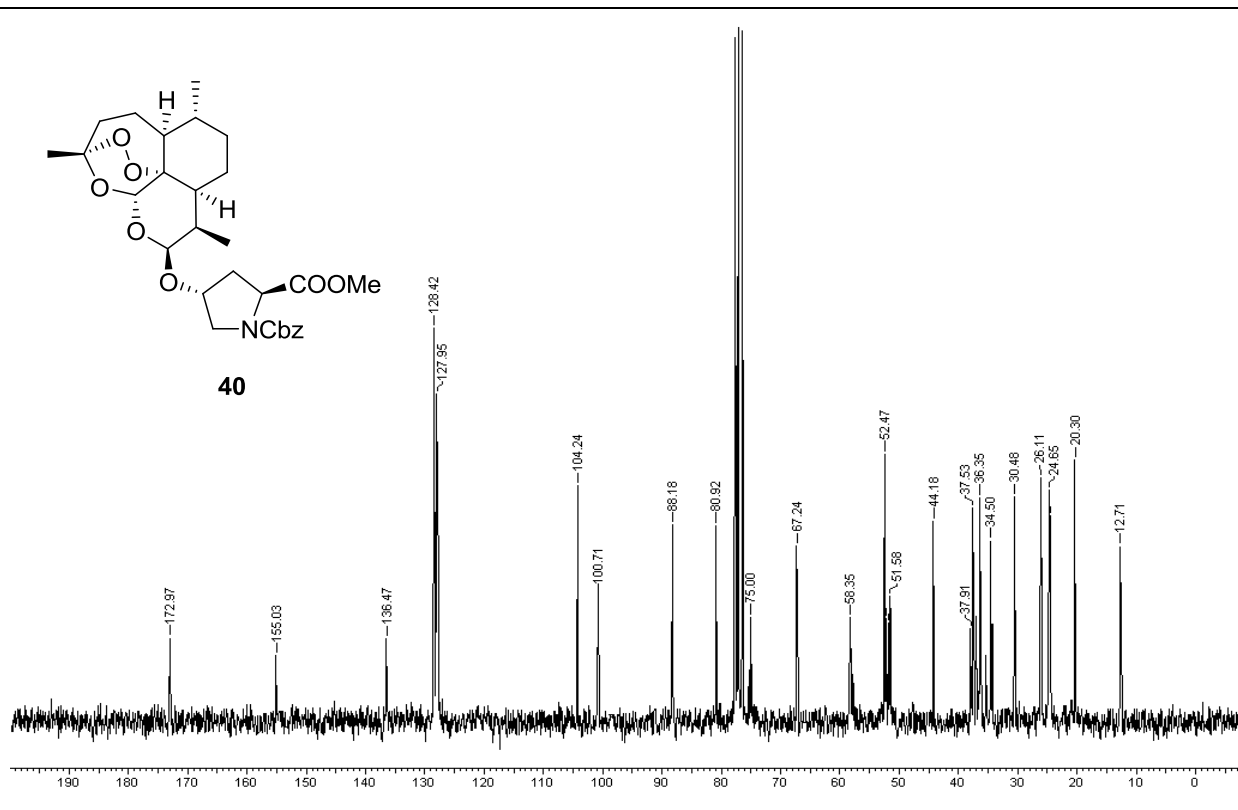
<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **43**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **43**

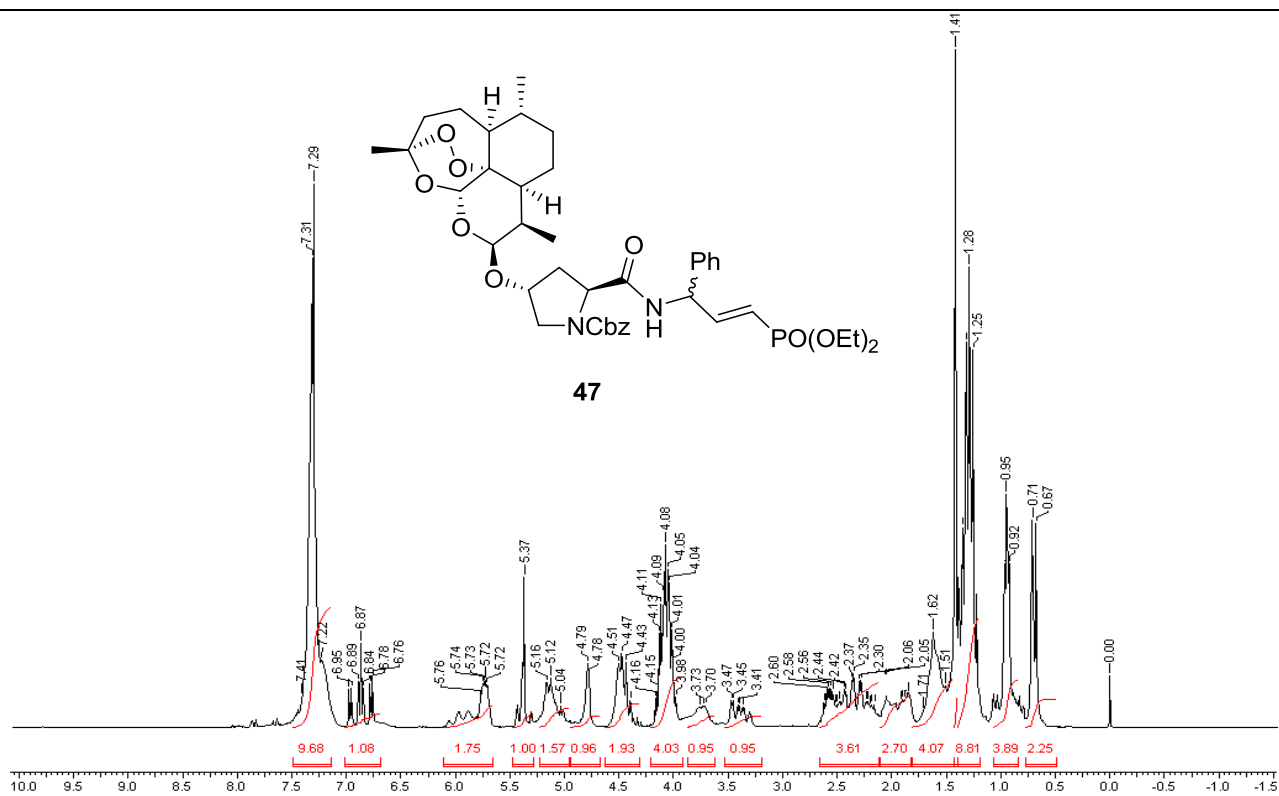
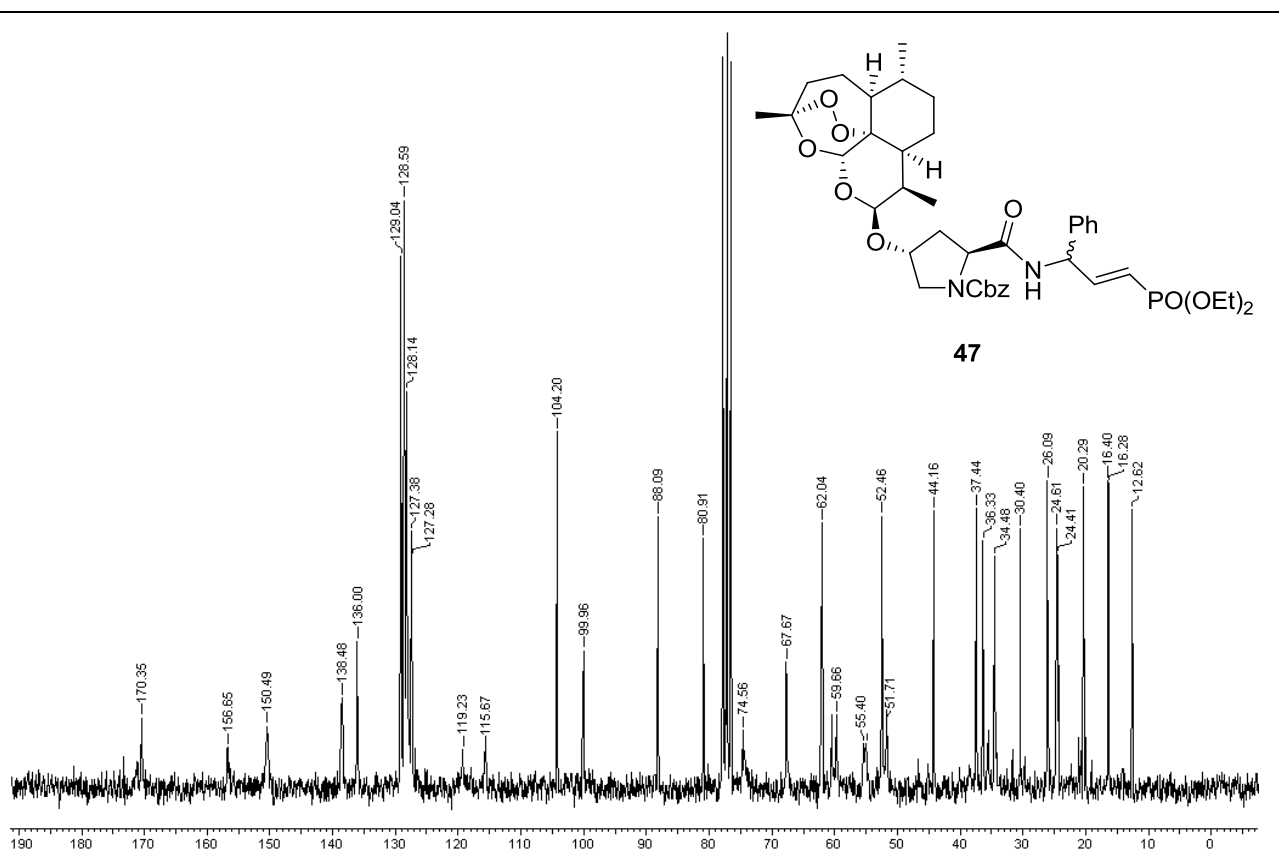
<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **44**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **44**

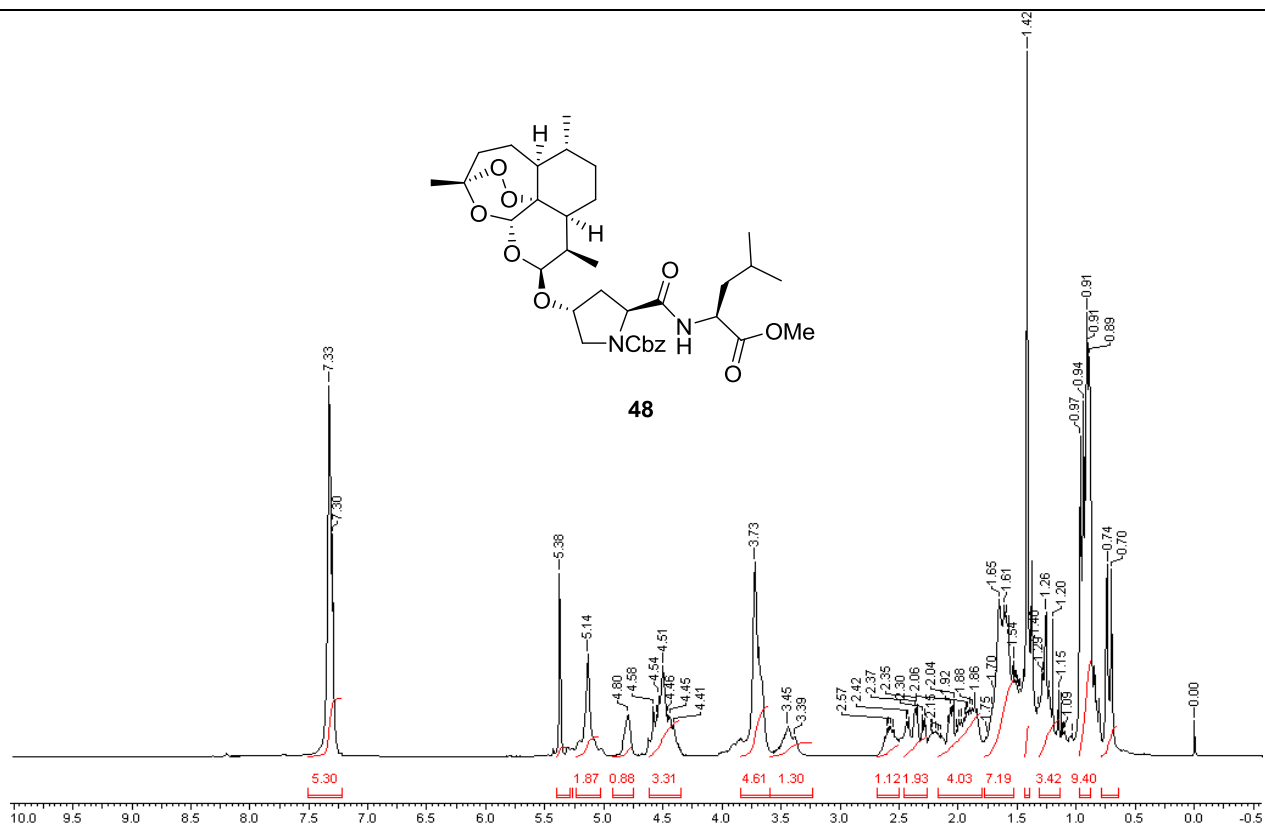
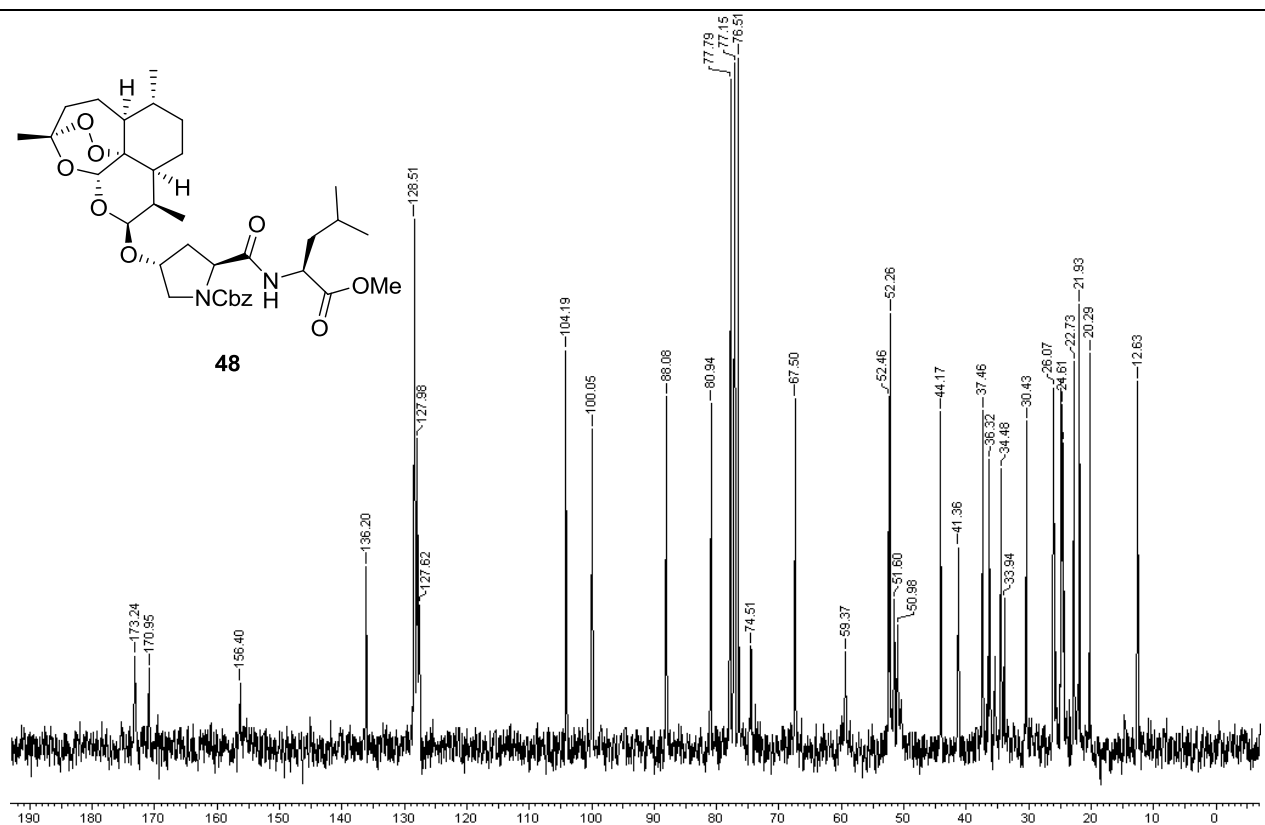
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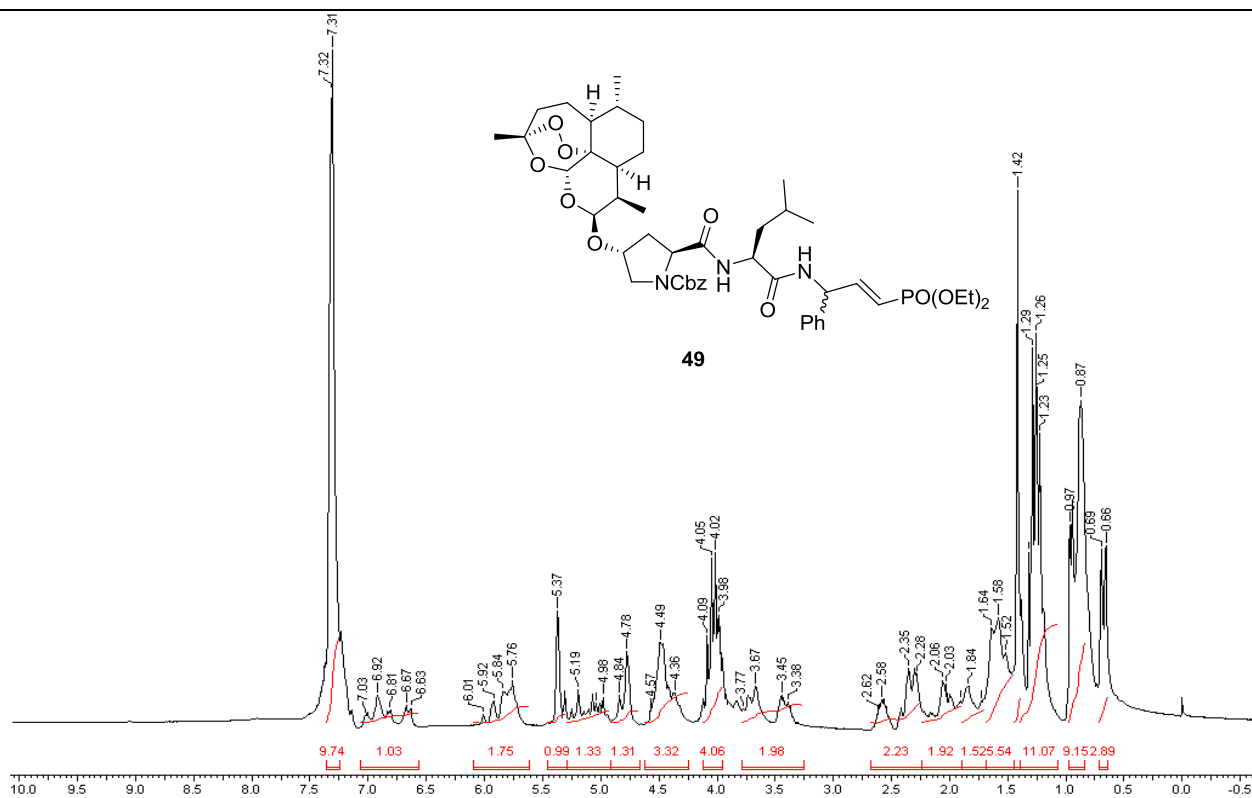
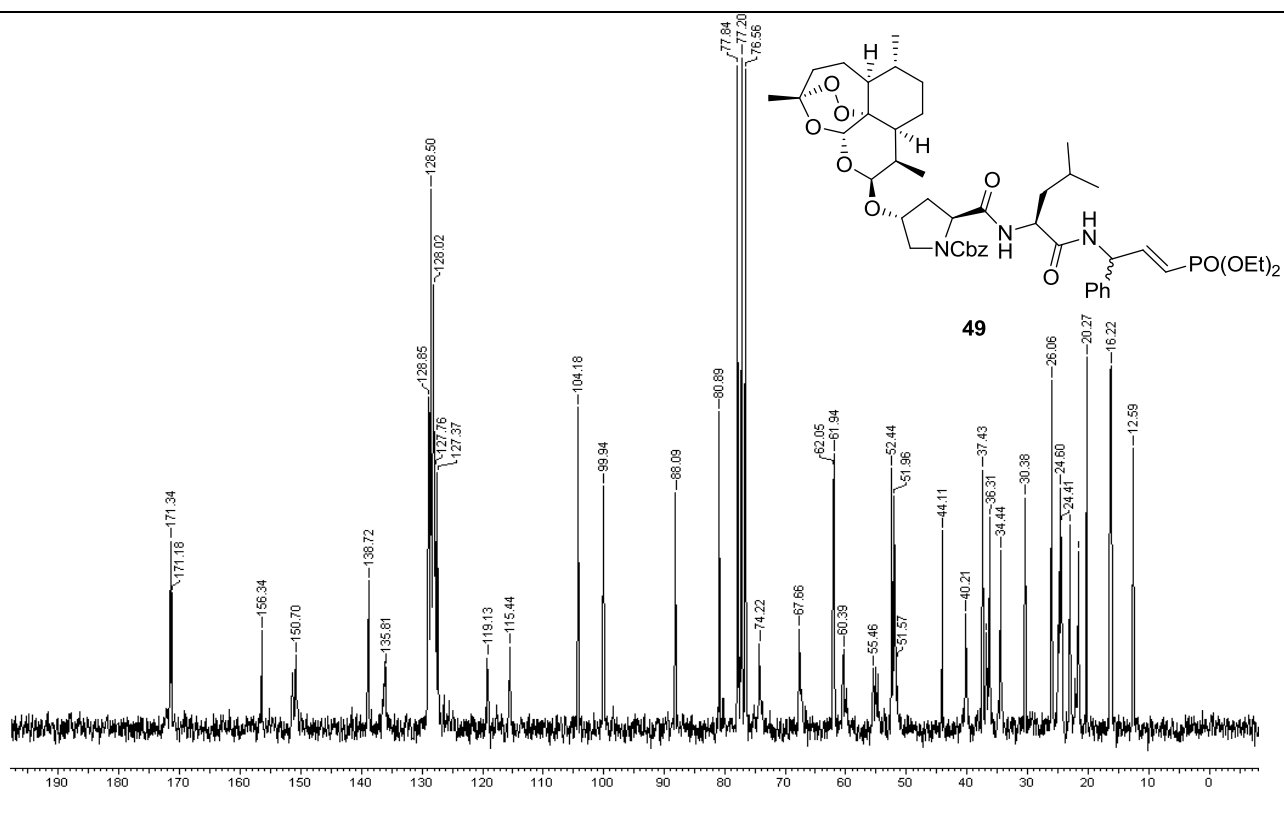
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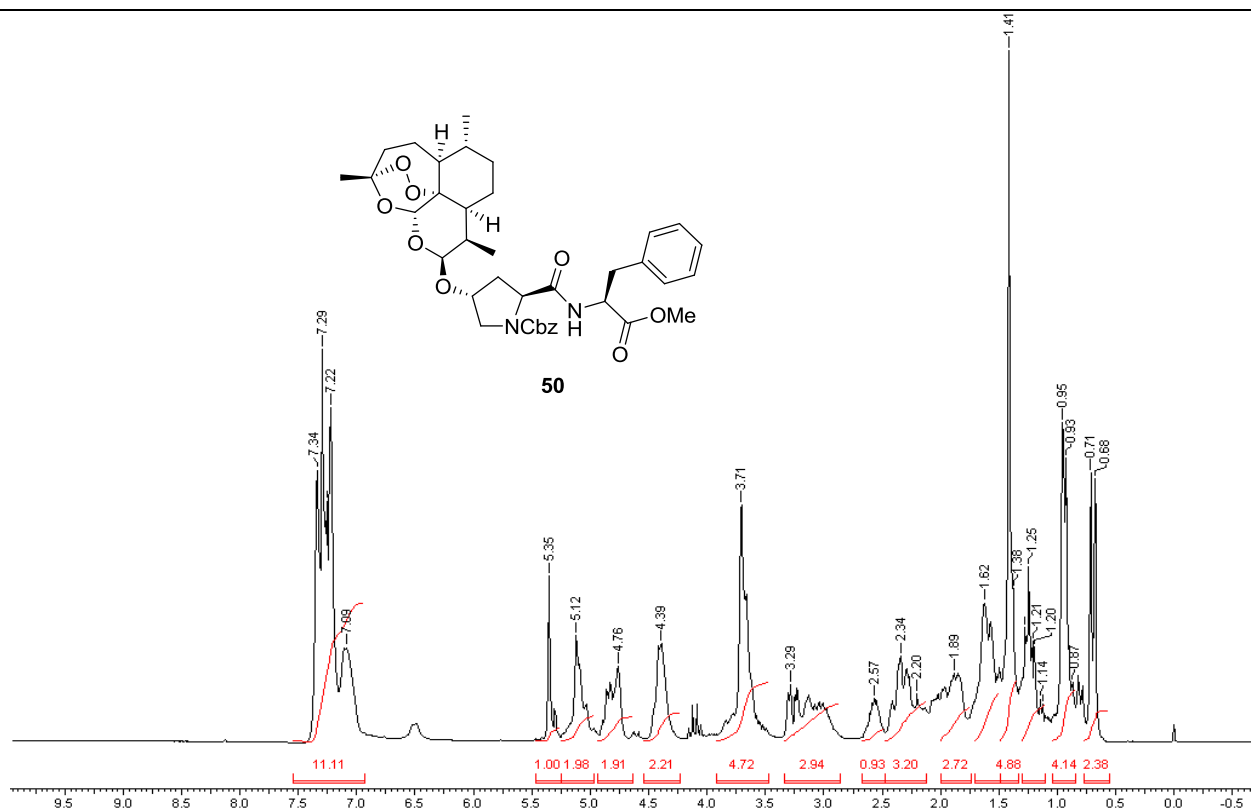
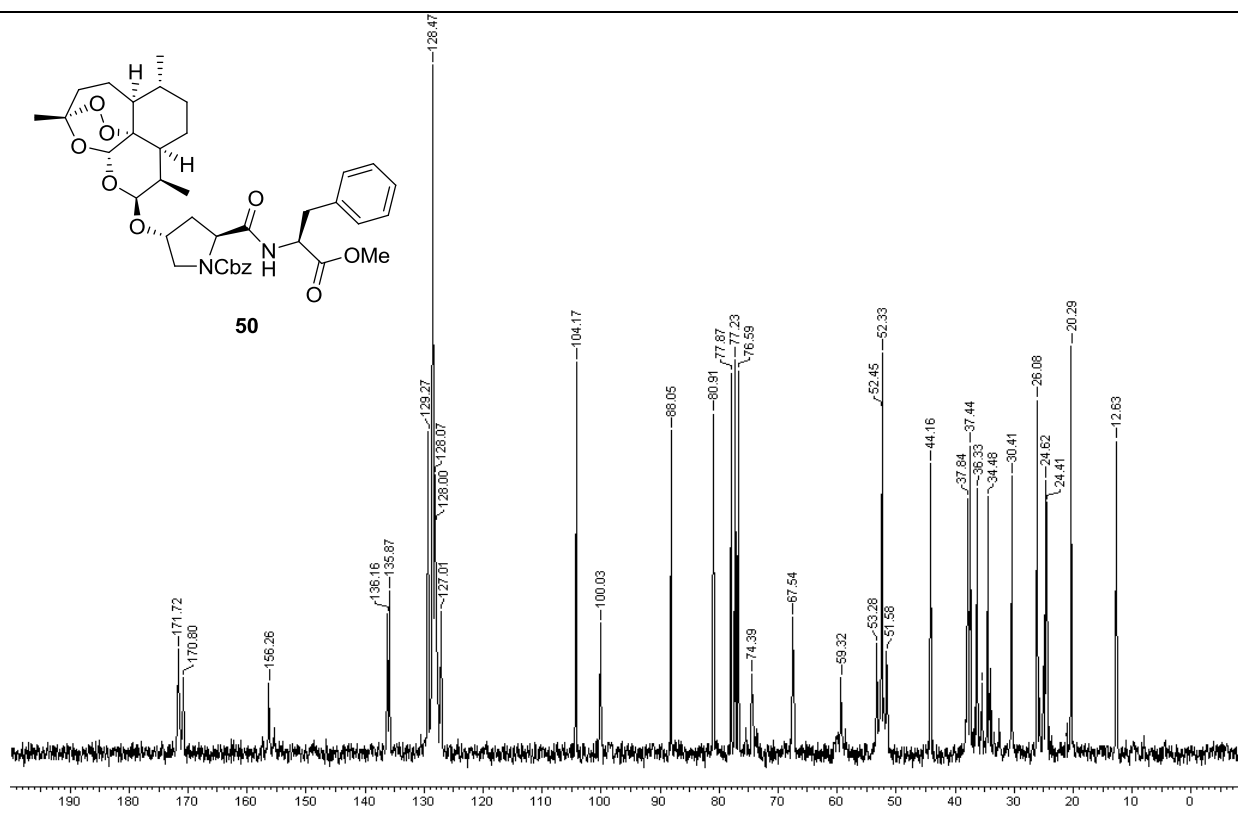


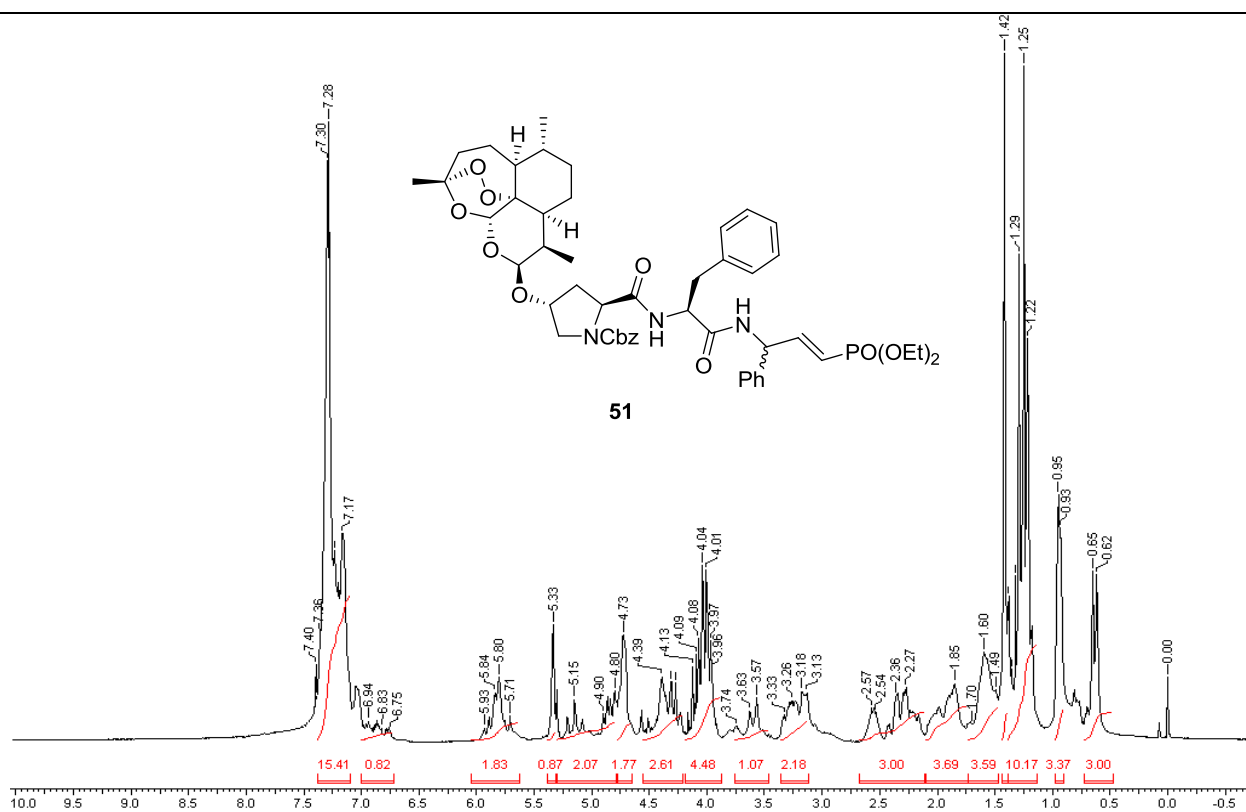
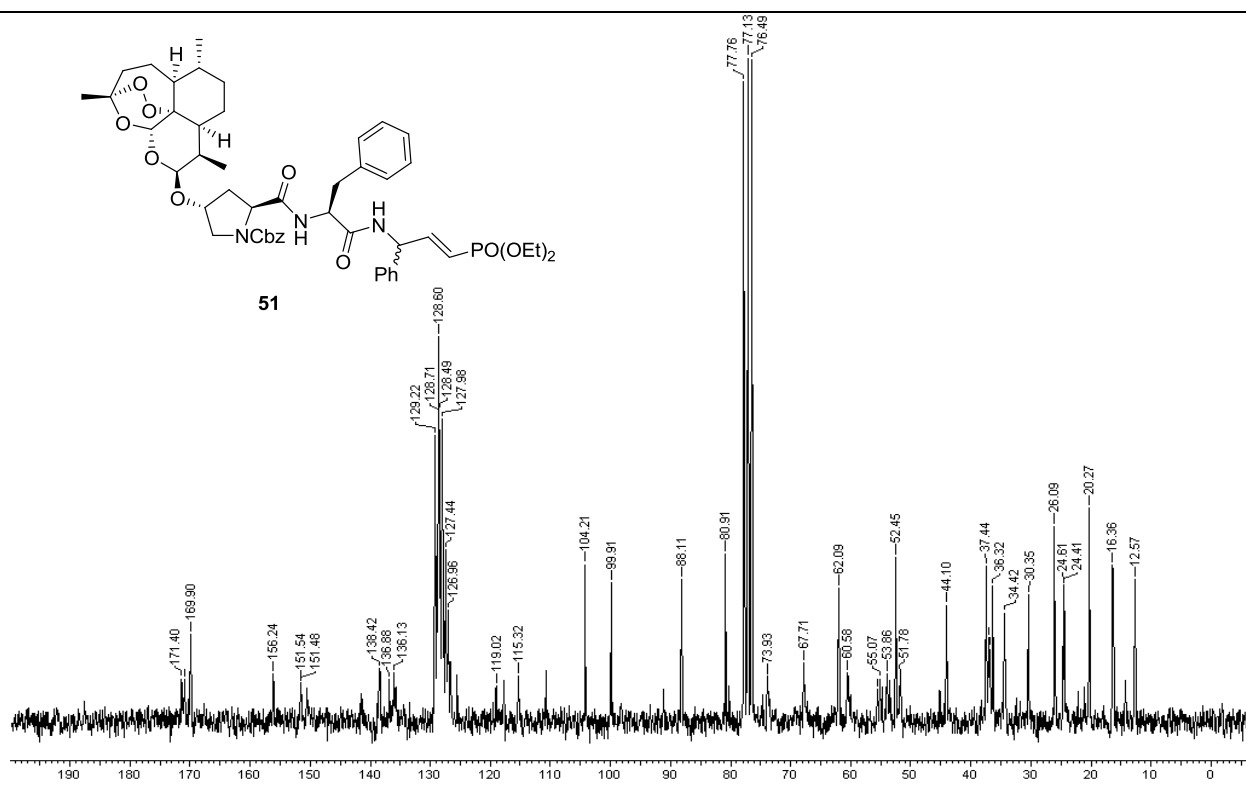
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **40** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **40**

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **47** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **47**

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **48** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **48**

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **49**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **49**

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **50** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **50**

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **51**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **51**

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## **Chapter 4**

### **Development of New Synthetically Useful Methodologies**

#### **Section A**

**An Efficient Synthesis of  
Benzodiazepinylphosphonates as Clostripain Inhibitors  
via FeCl<sub>3</sub>-Catalyzed Four-Component Reaction**

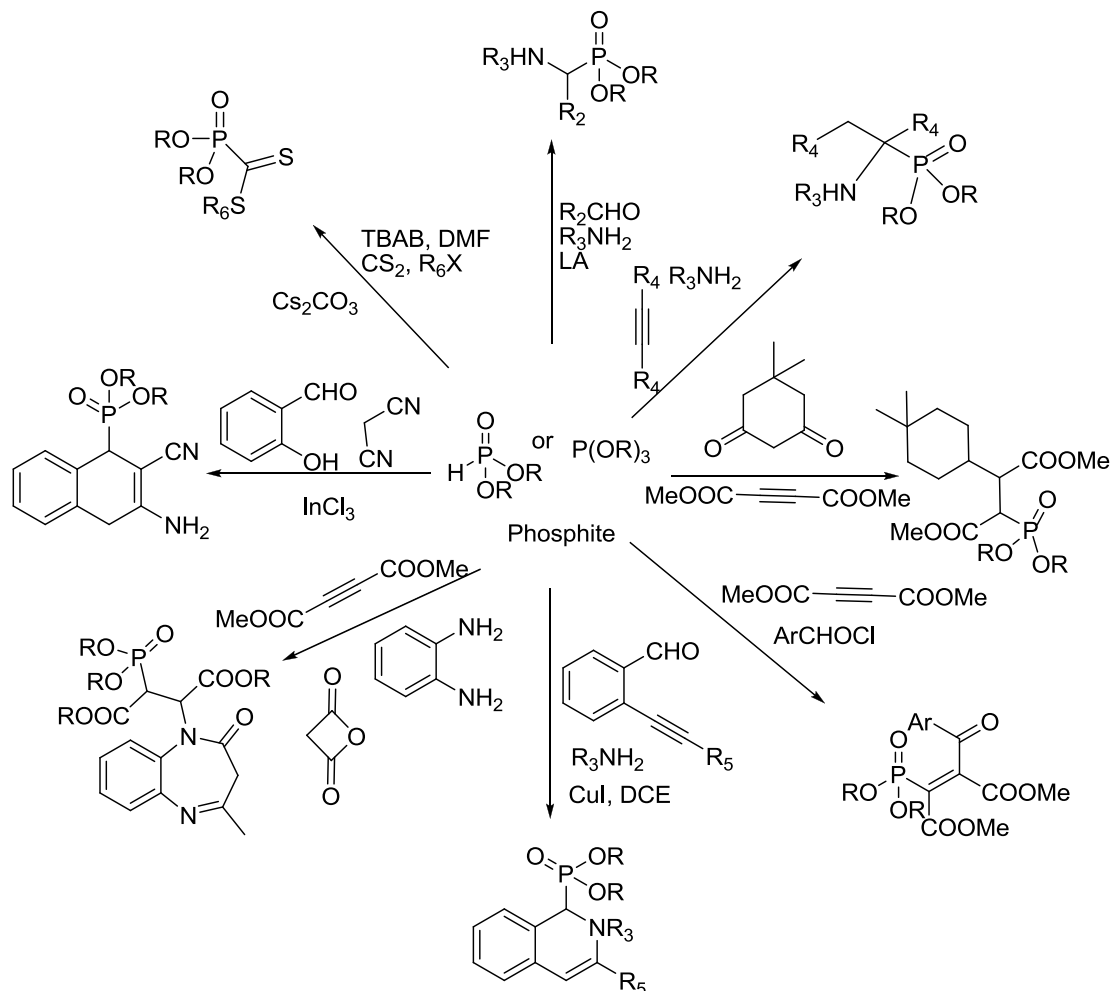
## Introduction

The need of production of large collections of diverse and structurally distinct compounds has led synthetic chemists to use extensively parallel automated synthesis or combinatorial chemistry.<sup>1</sup> This fact permitted the concept of diversity-oriented synthesis<sup>2</sup> and their use for the exploration of reaction pathways in cells and organisms leading eventually to the identification of therapeutic protein targets in a systematic way.<sup>2</sup> However, in nearly all cases the strategy to produce a compound has been divergent, that is, only two reagents react in every step of the synthesis. As a contrast to this multistep strategy, a new concept for the synthesis of a target or library with a higher chemical efficiency, the multicomponent reactions has emerged.<sup>3</sup> Even though the history of MCRs dates back to the second half of 19th century with reactions like Strecker, Hantzsch and Biginelli, it was only in the last decades with the work of Ugi and co-workers that the concept of multicomponent reactions has emerged as a powerful tool in synthetic chemistry.<sup>4</sup>

In MCR, more than two reactants are reacted in a reaction flask to furnish a product that incorporates substantial portions of all the components.<sup>5</sup> In its true form, MCR involves formation of several bonds in single operation without the need for isolation of intermediates formed, changing the reaction conditions or adding further reagents. In recent years, multicomponent reactions (MCRs) have gained tremendous attention of medicinal as well as organic chemists for the generation of compound libraries of novel chemical entities to satisfy the need of high-throughput screening for new bioactive molecules having diversified scaffolds.<sup>6</sup> Therefore, design and development of novel MCRs are the current need of both academia and industry. The multicomponent variant also has following added bonuses: (a) it is more convergent than the uni- and bi-molecular domino processes, (b) the structure of the reaction product is easily diversified by systematic variation of each input, (c) the starting materials are either commercially available or easily prepared and (d) the number of theoretically accessible compounds are extremely large.

Many important name reactions are MCR in nature<sup>4a,7</sup> *e.g.* Strecker, Hantzsch, Biginelli, Mannich, Passirini, Ugi reactions etc. Some classes of compounds such as

isonitrile and 1,3-dicarbonyl compounds have found wide applications in a variety of MCRs. Similarly alkyl/aryl phosphites have also been utilized as an important participating component in some MCRs as depicted in Scheme 1.<sup>8</sup>

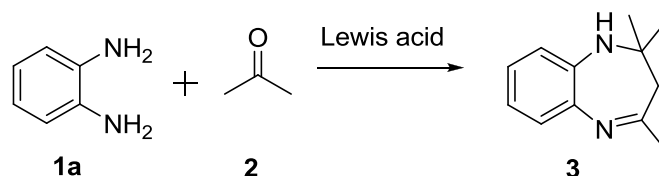


**Scheme 1.** Reported one pot reaction involving phosphites

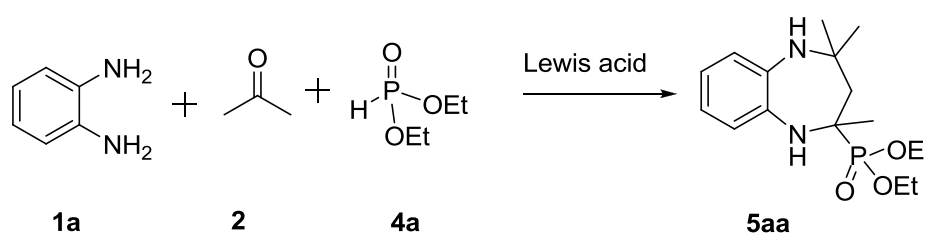
## Present Work

We directed our efforts to develop a new one-pot reaction involving phosphite as one of the reactant. Since phosphites are known to attack on imine as used in Kabachnik-Field reaction, the same concept could be employed to develop a new reaction in which imine is the final product. Recently, Bhattacharya *et al* described three-component reaction of aldehydes, amines, and diethyl phosphite catalyzed by Amberlite IR 120 (acidic)<sup>8f</sup> or bismuth nitrate<sup>8g</sup> affording the corresponding  $\alpha$ -amino phosphonates in excellent yields. In the literature,

syntheses of benzodiazepines have been accomplished by reacting *o*-phenylenediamine and ketones catalyzed by various Lewis acids (Scheme 2).<sup>9</sup> We envisaged that further nucleophilic attack of phosphite on the imine would result in the formation of BDPs in one-pot in a true MCR fashion (Scheme 3).



**Scheme 2.** Reported one-pot synthesis of benzodiazepine.



**Scheme 3.** Proposed one-pot synthesis of benzodiazepinyl phosphonate.

The benzodiazepines represent a biologically active class of compounds which exhibits wide range of therapeutic and pharmacological properties<sup>10</sup> such as anticonvulsant, anti-anxiety, analgesic, hypnotic, sedative, antidepressant, antiinflammatory agents, inhibitor of hepatitis C NS5B RNA polymerase,<sup>11</sup> antagonists of platelet-activating factor, psychotropic activity, caspase-1 inhibitors,<sup>12</sup> antitumor agents<sup>10</sup> and  $\beta$ -secretase inhibitors.<sup>10</sup>  $\alpha$ -Aminophosphonates have also shown various biological activities such as peptide mimics,<sup>13</sup> haptens of catalytic antibodies,<sup>14</sup> antibiotics and pharmacological agents<sup>15</sup> and herbicides.<sup>16</sup> Therefore, we opined that benzodiazepinyl phosphonates (BDPs) will be an interesting class of compounds as it combines these two biologically active moieties and also the synthesis of benzodiazepinyl phosphonates could be achieved in one-pot utilizing the MCR. Further,  $\alpha$ -aminophosphonates are considered to be the structural analogues of the corresponding  $\alpha$ -amino acids and transition-state mimics of peptide hydrolysis, the phosphonate group of  $\alpha$ -aminophosphonates can act as an electrophile which is the common requirement of cysteine protease inhibitors.<sup>17</sup> This generates the possibility that BDPs can act as cysteine protease inhibitor. Clostripain is one of the cysteine protease associated with collagenase,

isolated from *Clostridium histolyticum* is an anaerobic rod-shaped, spore forming bacillus, which belongs to a group of *Clostridium* spp<sup>18</sup> causing deadly gas gangrene, a severe pathologic condition. These *clostridium* species are also responsible for various disorders like pseudomembranous colitis, food poisoning, tetanus and enteroxemia. Therefore, inhibitors of clostripain could be utilized in the therapy of gas gangrene.

## Results and Discussion

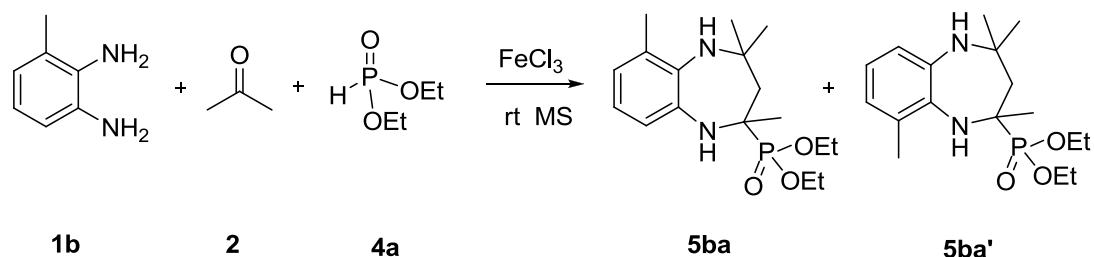
Initially the reaction of *o*-phenylenediamine **1a**, acetone **2** and diethylphosphite **4a** was carried out in presence of silica-perchloric acid however; BDP **1aa** was obtained in low yield (12%) even after 2 days. The slow reaction and low yield could be attributed to the less electrophilic ketimine which results in the slow attack of the phosphite. To overcome this problem, we screened several catalysts and reaction conditions as shown in Table 1. Catalyst screening and reaction condition optimization revealed that FeCl<sub>3</sub> was the best catalyst and use of molecular sieves further reduced the reaction time greatly. After optimizing the reaction condition, we carried out generalization of this reaction by reacting structurally diverse diamines, ketone and phosphites. The results are summarized in Table 2.

**Table 1.** Catalyst screening and reaction condition optimization for one-pot synthesis of benzodiazepinyl phosphonate.

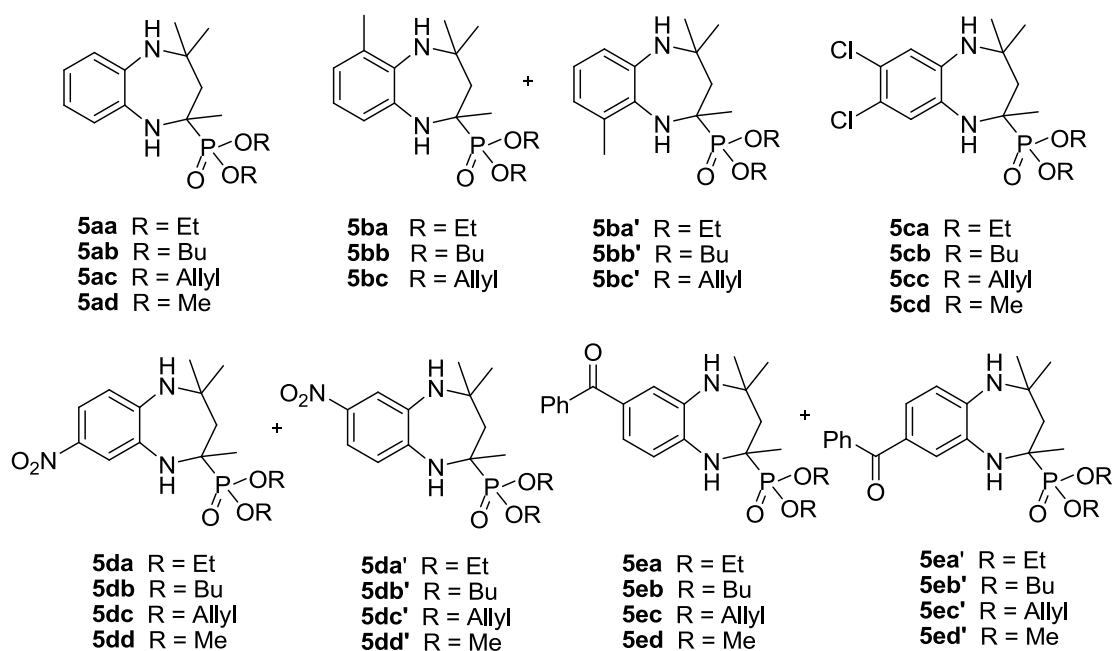
Sl. No.	Catalyst	Condition	Time	Yield (%)
1	HClO <sub>4</sub> -SiO <sub>2</sub> <sup>19</sup>	RT	2 day	12
2	Mg(ClO <sub>4</sub> ) <sub>2</sub>	RT	2 day	15
3	TaCl <sub>5</sub> -SiO <sub>2</sub> <sup>20</sup>	RT	2 day	23
4	FeCl <sub>3</sub>	Reflux	6 hr	decomposition
5	FeCl <sub>3</sub>	Microwave	1 min	decomposition
6	FeCl <sub>3</sub>	RT	2 day	43 %
7	FeCl <sub>3</sub>	RT, MS 4 Å	1 hr	63 %



Different substituted diamines underwent one-pot reaction to yield corresponding benzodiazepinyl phosphonates (Figure 1). In case of substituted benzene-1,2-diamine, two regioisomers were formed however, which could not be separated by repeated chromatography (Scheme 4). Thus, ratio of the regioisomers was calculated from  $^1\text{H}$  NMR *e.g.* in case of 3-methyl-1, 2-diamine **1b**, two regioisomers **5ba** and **5ba'** were formed in the ratio of 1:3 (see experimental).



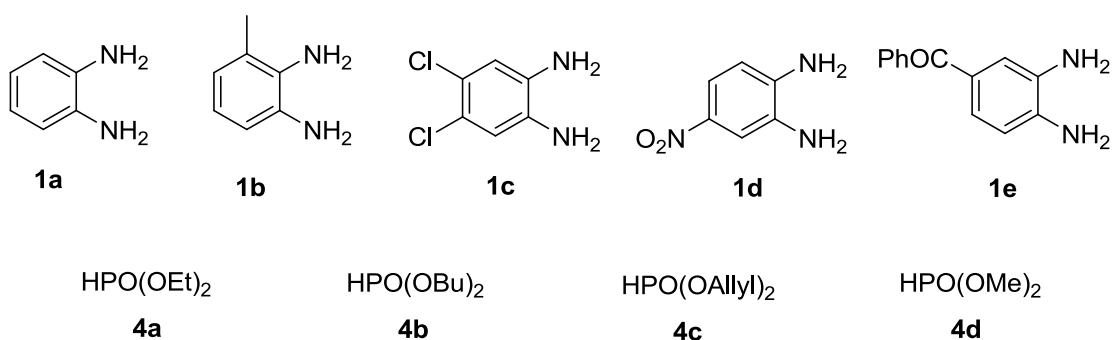
**Scheme 4.** One pot synthesis of benzodiazepinyl phosphonate from substituted diamine: 3-methylbenzene-1,2-diamine forming two regioisomers.



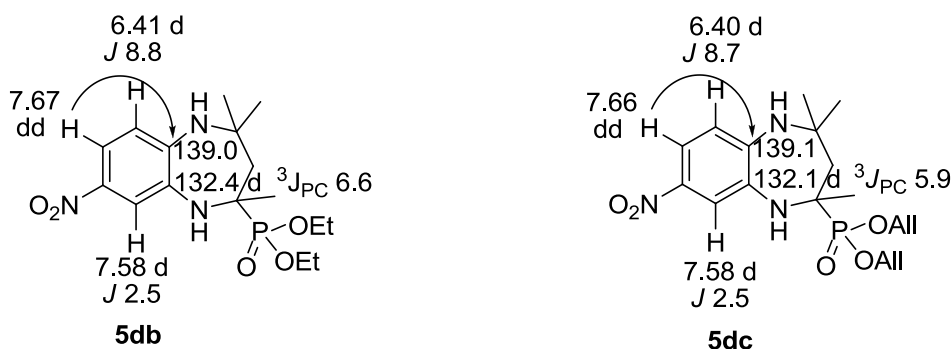
**Figure 1.** Structures of benzodiazepinyl phosphonates synthesized through one-pot reaction.

**Table 2.** One pot synthesis of benzodiazepinyl phosphonate catalysed by FeCl<sub>3</sub>.

Entry	Amine	Phosphite	Product code	Yield (%) <sup>a</sup>	Ratio of isomers <sup>b</sup>
1	1a	4a	5aa	63	-
2	1a	4b	5ab	63	-
3	1a	4c	5ac	66	-
4	1a	4d	5ad	65	-
5	1b	4a	5ba+5ba'	42	1:6
6	1b	4b	5bb+5bb'	50	1:8
7	1b	4c	5bc+5bc'	52	1:5
8	1c	4a	5ca	59	-
9	1c	4b	5cb	55	-
10	1c	4c	5cc	52	-
11	1c	4d	5cd	51	-
12	1d	4a	5da+5da'	63	1:8
13	1d	4b	5db+5db'	59	1:0
14	1d	4c	5dc+5dc'	57	1:0
15	1d	4d	5dd+5dd'	53	1:1
16	1e	4a	5ea+5ea'	43	3:4
17	1e	4b	5eb+5eb'	42	1:3
18	1e	4c	5ec+5ec'	50	1:2
19	1e	4d	5ed+5ed'	49	1:1

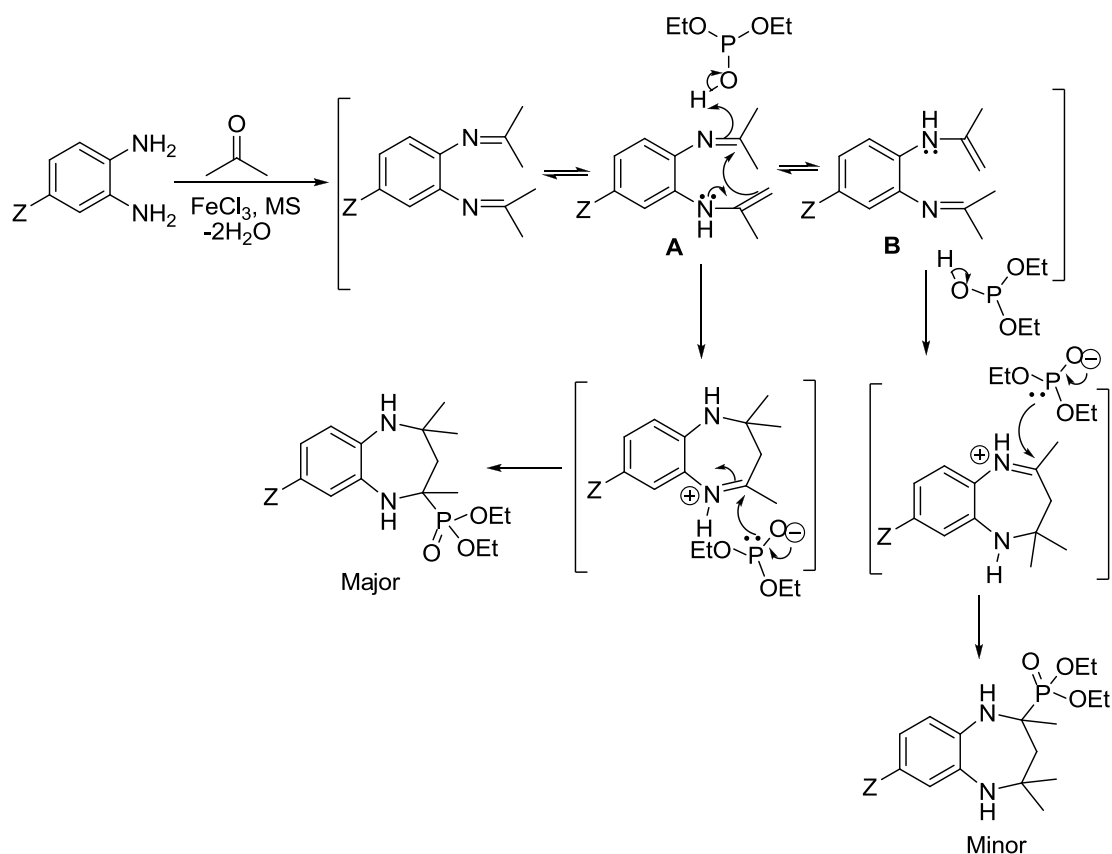
<sup>a</sup>Yields refer to the isolated yields.<sup>b</sup>Ratio of regioisomers formed are calculated based on <sup>1</sup>H NMR spectra.

In case of reaction of butylphosphite and allylphosphite with nitro-substituted diamine (entry 13 & 14, respectively) exclusively one regioisomer was formed. The characterization of the regioisomers was based on HMBC correlation as shown in the Figure 2. In compound **5db**, the aromatic proton at  $\delta$  7.67 (dd, 1H) showed HMBC correlation with carbon at  $\delta$  139.0 which in turn did not show any C-P coupling suggesting *syn*-structure to the regioisomer **5db**. Similarly in case of **5dc**, the proton at  $\delta$  7.66 (dd, 1H) showed HMBC correlation with carbon at  $\delta$  139.1 which also did not show any C-P coupling thereby suggesting *syn*-structure to the **5dc**.



**Figure 2.** HMBC correlations of **5db** and **5dc**.

The above results indicate that in case of unsymmetrical diamines, the major product formed is *syn*-regioisomer. Further, this could be explained on the basis of plausible reaction mechanism shown in the Scheme 5. Initially, di-imine is formed by the reaction of ketone with di-amine catalyzed by  $\text{FeCl}_3$ . The di-imine exists in two tautomeric forms **A** and **B**. The tautomeric form **A** is more reactive than **B** since lone pair of electrons on nitrogen in **B** is conjugated with the electron-withdrawing group Z and therefore not easily available for the donation while the lone pair of electrons on nitrogen in **A** is not conjugated with the electron-withdrawing Z group and thereby easily donated leading to the intermediate that gives the *syn*-regioisomer as the major product. Similarly, the reaction of tautomeric form **B** gives *anti*-regioisomer as the minor product.



**Scheme 5.** Plausible mechanism for the formation benzodiazepinyl phosphonates (major and minor regioisomers).

Several ketones such as cyclobutanone, ethyl methyl ketone, isopropyl methyl ketone and acetone were employed however, only acetone furnished the corresponding products, BDPs. In case of other ketones, only intermediate ketimines were formed. This could be due to the steric factor. With higher ketones, the ketimines formed are so sterically crowded that it could not allow phosphite to attack even when the reaction was continued for a prolonged period of time. All the phosphites employed worked well except triphenyl phosphite and diphenyl phosphite. This can also be explained on the basis of steric factor.

### ***In vitro* Inhibition Activity of BDP against Clostripain**

Clostripain is one of the cysteine protease associated with collagenase which is isolated from *Clostridium histolyticum* bacillus, which belongs to a group of *Clostridium* spp.<sup>18</sup> These *clostridium* species are also responsible for various disorders like pseudomembranous colitis, food poisoning, tetanus, enteroxemia and deadly gas

gangrene. Therefore inhibitors of clostripain could be used in the therapy of gas gangrene. The first report on this bacterium was found in manuscripts of Greek physician Hippocrates, where he described a disease that can be diagnosed as gas gangrene. *C. histolyticum* is also known as an etiological agent of necrotizing fasciitis and other serious complications.

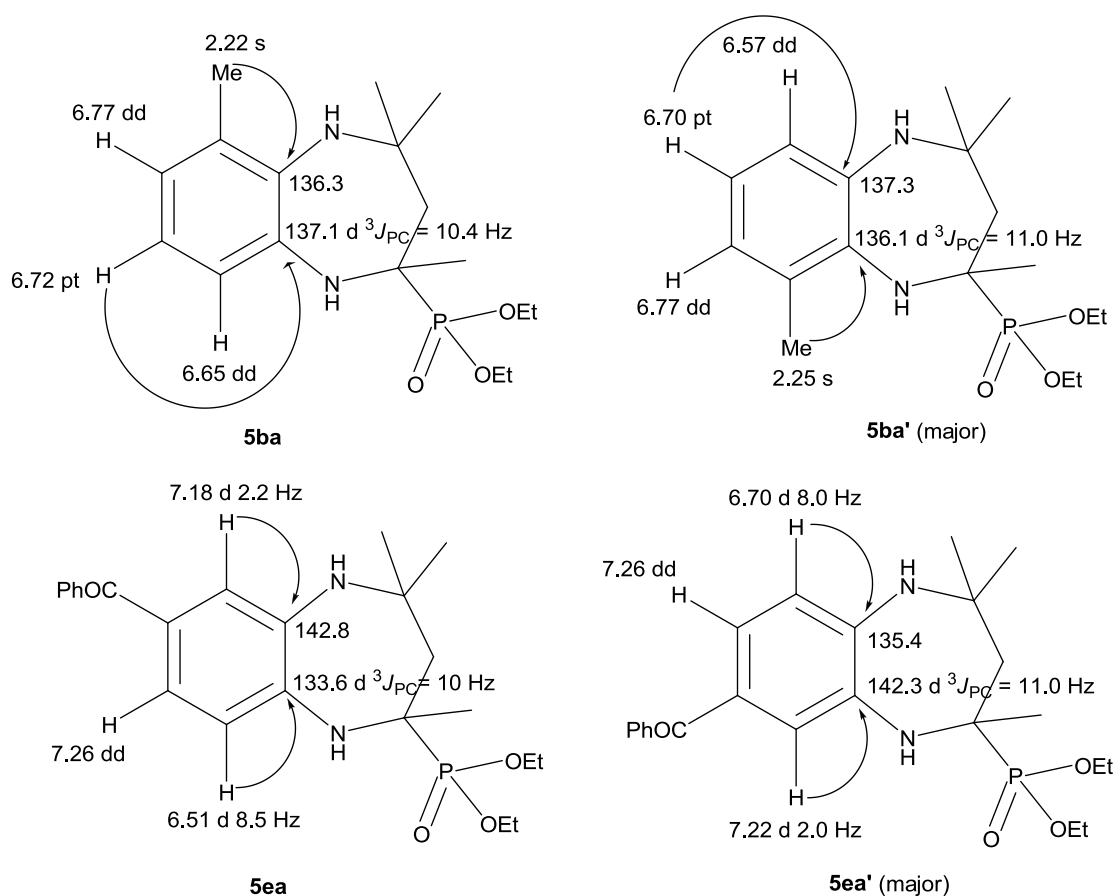
The synthesized benzodiazepinyl phosphonates were tested for their *in vitro* inhibition of clostripain and the results are summarized in Table 3. The compound **5ba+5ba'** derived from 2,3-diamino toluene and diethyl phosphite inhibited the clostripain enzyme with IC<sub>50</sub> value of 32 μM. When the phosphite was changed to allyl (**5bc+5bc'**) and butyl (**5bb+5bb'**) activity dropped to 80 and 278 μM, respectively. Similar trend in activity profile was observed in case of benzodiazepinyl phosphonate obtained by the reaction of 3,4-diamino benzophenone. Compound **5ea+5ea'** showed IC<sub>50</sub> value of 36 μM which dropped to 70 and 140 μM in case of **5ec+5ec'** and **5eb+5eb'**, respectively. The benzodiazepinyl phosphonates derived from symmetrical diamines (**1a** and **1c**) were found to be comparatively less potent than their corresponding benzodiazepinyl phosphonates derived from unsymmetrical diamines (**1b**, **1d** and **1e**).

**Table 3.** Inhibition activities of BDPs against clostripain.

Entry	Compound Code	IC <sub>50</sub> values (μM)	Entry	Compound Code	IC <sub>50</sub> values (μM)
1	<b>5aa</b>	ND	11	<b>5cd</b>	150
2	<b>5ab</b>	780	12	<b>5da+5da'</b>	>400
3	<b>5ac</b>	>2000	13	<b>5db+5db'</b>	ND
4	<b>5ad</b>	490	14	<b>5dc+5dc'</b>	165
5	<b>5ba+5ba'</b>	32	15	<b>5dd+5dd'</b>	220
6	<b>5bb+5bb'</b>	278	16	<b>5ea+5ea'</b>	36
7	<b>5bc+5bc'</b>	80	17	<b>5eb+5eb'</b>	140
8	<b>5ca</b>	>300	18	<b>5ec+5ec'</b>	70
9	<b>5cb</b>	175	19	<b>5ed+5ed'</b>	90
10	<b>5cc</b>	>300			

## Separation and Characterization of Regioisomers

The activity data revealed that many of the BDPs showed inhibition activity against clostripain. Mixture of regio-isomers **5ba**+**5ba'** and **5ea**+**5ea'** have shown good inhibition activity against clostripain with  $IC_{50}$  value of 32 and 36  $\mu$ M, respectively. Therefore, separations of the regioisomers were attempted. After trying several solvent systems on TLC, we were able to develop a solvent system consisting of petroleum ether: ethyl acetate: formic acid in the ratio of 4:5:1 for the separation of regioisomers on preparative TLC. The assignment of both the regioisomers was on the basis of the HMBC correlations.



**Figure 2.** HMBC of the **5ba**, **5ba'**, **5ea** and **5ea'**

One of the two quaternary carbons of the fused ring showed doublet due to the C-P three bond coupling and this enabled us to assign which one is near the phosphorus atom. In case of **5ba** and **5ba'** the methyl protons were helpful to assign the structure to the regioisomers. In **5ba**, the methyl proton at  $\delta$  2.22 showed HMBC correlation with carbon at  $\delta$  136.3 which is not coupled to phosphorus indicating anti-

structure **5ba**. But in case of **5ba'**, the methyl proton at  $\delta$  2.25 showed HMBC correlation with  $\delta$  136.1 which is coupled to phosphorus indicating *syn*-structure **5ba'**. On the similar ground, **5ea** and **5ea'** regioisomers were assigned *anti*- and *syn*-structure, respectively. In **5ea**, the proton at  $\delta$  7.18 (d) with lower coupling constant ( $J = 2.2$  Hz) showed HMBC correlation with quaternary carbon at  $\delta$  142.8 which showed no further splitting due to C-P coupling indicating *anti*-structure to the **5ea**. In case of **5ea'**, proton at  $\delta$  7.22 (d) with lower coupling constant ( $J = 2.0$  Hz) showed HMBC correlation with carbon at  $\delta$  142.3 which showed further splitting due to C-P coupling indicating *syn*-structure to **5ea'**.

The clostripain inhibition activities of these separated BDPs were further carried out and results are summarised in Table 4. The activity profile of the separated regioisomers indicates that the major regioisomers are more active than their corresponding minor regioisomers.

**Table 4.** Inhibition activity of separated regioisomers of BDP against clostripain.

Entry	Compound Code	IC <sub>50</sub> values ( $\mu$ M)
1	<b>5ba</b>	>300
2	<b>5ba'</b>	28
3	<b>5ea</b>	>300
4	<b>5ea'</b>	10

## Conclusions

In summary, we have developed a four-component reaction of diamine, ketone and phosphite catalyzed by FeCl<sub>3</sub> to generate novel chemical entities, benzodiazepinyl phosphonates (BDPs). All the synthesised compounds were assayed *in vitro* for their efficacies against clostripain, a disease model for gas gangrene. Some of the synthesized BDPs showed remarkable cysteine protease inhibition activities in the micro molar range thereby suggesting that these chemical entities could be further explored for their protease inhibition to obtain a lead compound.

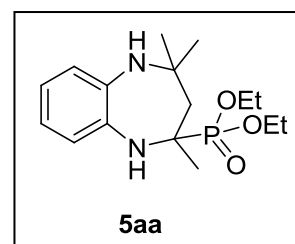
## Experimental

### Chemistry

**Typical experimental procedure:** To a mixture of *o*-phenylenediamine (1 mmol), acetone (0.5 mL) and molecular sieves 4 Å (50 mg), FeCl<sub>3</sub> (10 mol%) and phosphite (1 mmol) were added. The reaction mixture was stirred at rt for 1 h. After completion of the reaction (TLC), saturated aq. NaHCO<sub>3</sub> (10 mL) was added to the reaction mixture and the product was extracted with EtOAc (3x20 mL). The combined organic layer was washed with brine (30 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuum to furnish the crude product which was purified by silica gel column chromatography by using ethyl acetate: petroleum ether (2:5 to 3:5) as eluant.

### Diethyl 2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5aa).

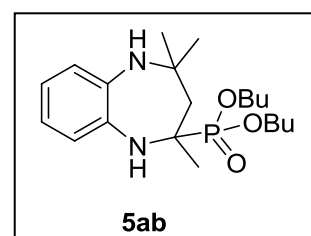
Yield: 63%; yellow syrup; IR (CHCl<sub>3</sub>): 3420, 3367, 3019, 1604, 1518, 1424, 1216, 1048, 1031 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 1.29-1.36 (m, 12H), 1.56 (d, <sup>3</sup>J<sub>PH</sub> = 17.2 Hz, 3H), 1.72-1.82 (m, 1H), 2.14-2.37 (m, 1H), 4.08-4.24 (m, 4H), 6.62-6.81 (m, 4H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ 16.5



(d, <sup>3</sup>J<sub>PC</sub> = 5.9 Hz), 16.6 (d, <sup>3</sup>J<sub>PC</sub> = 5.5 Hz), 23.5, 30.5, 33.6, 44.0 (d, <sup>2</sup>J<sub>PC</sub> = 2.6 Hz), 53.2 (d, <sup>3</sup>J<sub>PC</sub> = 14.6 Hz), 56.2 (d, <sup>1</sup>J<sub>PC</sub> = 148.2 Hz), 62.5 (d, <sup>2</sup>J<sub>PC</sub> = 7.7 Hz), 63.2 (d, <sup>2</sup>J<sub>PC</sub> = 7.0 Hz), 121.6, 121.8, 121.9, 122.0, 136.8 (d, <sup>3</sup>J<sub>PC</sub> = 12.4 Hz), 137.5; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 29.1; MS (ESI): *m/z* 327.29 (M+H)<sup>+</sup>, 349.28 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>16</sub>H<sub>27</sub>N<sub>2</sub>O<sub>3</sub>P: C, 58.88; H, 8.34; N, 8.58. Found: C, 58.95; H, 8.28; N, 8.63.

### Dibutyl 2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5ab).

Yield: 63%; yellow syrup; IR (CHCl<sub>3</sub>): 3421, 3020, 2972, 1599, 1476, 1423, 1216, 1030 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 0.91 (t, *J* = 7.3 Hz, 6H), 1.28 (s, 3H), 1.30 (s, 3H), 1.56 (d, <sup>3</sup>J<sub>PH</sub> = 17.2 Hz, 3H), 1.26-1.66 (m, 8H), 1.72-1.77 (m, 1H), 2.15-2.21 (m, 1H), 4.04-4.14 (m, 4H), 6.60-



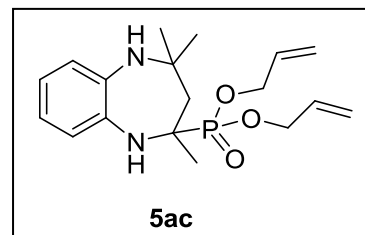
6.76 (m, 4H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 13.6, 18.7, 23.6, 30.6, 32.6 (d, <sup>3</sup>J<sub>PC</sub> = 5.8 Hz), 32.7 (d, <sup>3</sup>J<sub>PC</sub> = 5.5 Hz), 33.4, 43.9 (d, <sup>2</sup>J<sub>PC</sub> = 2.6 Hz), 53.2 (d, <sup>3</sup>J<sub>PC</sub> = 14.6 Hz),



56.4 (d,  $^1J_{PC} = 147.8$  Hz), 66.2 (d,  $^2J_{PC} = 8.0$  Hz), 66.8 (d,  $^2J_{PC} = 7.3$  Hz), 121.5, 121.6, 121.8, 121.9, 136.8 (d,  $^3J_{PC} = 11.7$  Hz), 137.4;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  29.77; MS (ESI):  $m/z$  383.32 ( $\text{M}+\text{H}$ ) $^+$ , 405.31 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{20}\text{H}_{35}\text{N}_2\text{O}_3\text{P}$ : C, 62.80; H, 9.22; N, 7.32. Found: C, 62.78; H, 9.34; N, 7.41.

**Diallyl 2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5ac).**

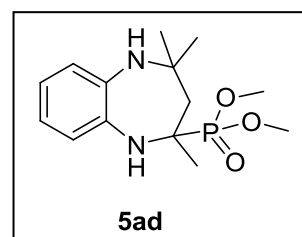
Yield: 66%; yellow syrup; IR ( $\text{CHCl}_3$ ): 3420, 3019, 2934, 1602, 1522, 1424, 1216, 1045  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.30 (s, 3H), 1.33 (s, 3H), 1.59 (d,  $^3J_{\text{PH}} = 17.4$  Hz, 3H), 1.74-1.84 (m, 1H), 2.18-2.31 (m, 1H), 4.52-4.65 (m, 4H), 5.14-5.43 (m, 4H), 5.83-6.03 (m, 2H),



6.61-7.01 (m, 4H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  23.7, 30.6, 33.5, 43.8 (d,  $^2J_{PC} = 2.2$  Hz), 53.2 (d,  $^3J_{PC} = 14.6$  Hz), 56.6 (d,  $^1J_{PC} = 147.1$  Hz), 66.9 (d,  $^2J_{PC} = 7.7$  Hz), 67.4 (d,  $^2J_{PC} = 6.9$  Hz), 118.0 (d,  $^4J_{PC} = 2.2$  Hz), 121.5, 121.8, 121.8, 122.0, 133.0 (d,  $^3J_{PC} = 5.9$  Hz), 133.2 (d,  $^3J_{PC} = 5.9$  Hz), 136.5 (d,  $^3J_{PC} = 11.7$  Hz), 137.5;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  30.43; MS (ESI):  $m/z$  351.27 ( $\text{M}+\text{H}$ ) $^+$ , 373.25 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{18}\text{H}_{27}\text{N}_2\text{O}_3\text{P}$ : C, 61.70; H, 7.77; N, 7.99. Found: C, 61.76; H, 7.69; N, 7.92.

**Dimethyl 2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5ad).**

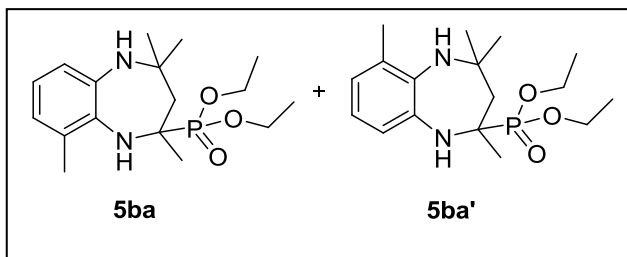
Yield: 65%; yellow syrup; IR ( $\text{CHCl}_3$ ): 3420, 3019, 2934, 1614, 1502, 1216, 1054  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.30 (s, 3H), 1.31 (s, 3H), 1.56 (d,  $^3J_{\text{PH}} = 17.3$  Hz, 3H), 1.75-1.80 (m, 1H), 2.04-2.37 (m, 1H), 3.79 (d,  $^3J_{\text{PH}} = 10.5$ , 3H), 3.81 (d,  $^3J_{\text{PH}} = 10.5$ , 3H), 6.64-6.66 (m, 1H), 6.72-6.74 (m,



1H), 6.77-6.80 (m, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  23.5, 30.3, 33.6, 44.0 (d,  $^2J_{PC} = 2.2$  Hz), 53.1 (d,  $^3J_{PC} = 14.6$  Hz), 53.2 (d,  $^2J_{PC} = 8.1$  Hz), 54.3 (d,  $^2J_{PC} = 7.3$  Hz), 56.6 (d,  $^1J_{PC} = 147.5$  Hz), 121.6, 121.8, 121.9, 122.2, 136.6 (d,  $^3J_{PC} = 12.5$  Hz), 137.5;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  32.27; MS (ESI):  $m/z$  299.23 ( $\text{M}+\text{H}$ ) $^+$ , 321.20 ( $\text{M}+\text{Na}$ ) $^+$ ; Anal. Calcd for  $\text{C}_{14}\text{H}_{23}\text{N}_2\text{O}_3\text{P}$ : C, 56.37; H, 7.77; N, 9.39. Found: C, 56.42; H, 7.70, N, 9.42.

**Diethyl 2,4,4,9-tetramethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5ba+5ba')** (1:6 regio-isomeric mixtures, only peaks corresponding to the major isomer are given).

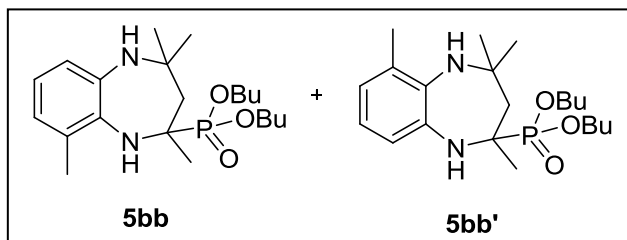
Yield: 42%; yellow syrup; IR (CHCl<sub>3</sub>): 3420, 3367, 3019, 1599, 1476, 1421, 1216, 1029 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.29-1.38 (m, 12H), 1.58 (d, <sup>3</sup>J<sub>PH</sub> = 17.9



Hz, 3H), 1.77-1.81 (m, 1H), 2.13-2.16 (m, 1H), 2.23 (s, 3H), 4.13-4.23 (m, 4H), 6.55 (d, *J* = 7.6 Hz, 1H), 6.66-6.69 (m, 1H), 6.74 (d, *J* = 7.6 Hz, 1H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 16.5 (d, <sup>3</sup>J<sub>PC</sub> = 5.5 Hz), 18.0, 23.4, 30.4, 33.4, 43.6 (d, <sup>2</sup>J<sub>PC</sub> = 1.8 Hz), 52.8 (d, <sup>3</sup>J<sub>PC</sub> = 15.4 Hz), 56.0 (d, <sup>3</sup>J<sub>PC</sub> = 148.0 Hz), 62.7 (d, <sup>2</sup>J<sub>PC</sub> = 8.2 Hz), 62.8 (d, <sup>2</sup>J<sub>PC</sub> = 7.3 Hz), 120.4, 120.9, 124.1, 128.2, 136.0 (d, <sup>2</sup>J<sub>PC</sub> = 10.9 Hz), 137.3; <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>): δ 30.30; MS (ESI): *m/z* 341.57 (M+H)<sup>+</sup>, 363.57 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>17</sub>H<sub>29</sub>N<sub>2</sub>O<sub>3</sub>P: C, 59.98; H, 8.59; N, 8.23. Found: C, 59.83; H, 8.63; N, 8.34.

**Dibutyl 2,4,4,9-tetramethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5bb+5bb')** (1:8 regio-isomeric mixtures, only peaks corresponding to the major isomer are given).

Yield: 50%; yellow syrup; IR (CHCl<sub>3</sub>): 3420, 3360, 3019, 1599, 1518, 1476, 1424, 1215, 1022 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 0.88-0.98 (m, 6H), 1.25-1.73 (m,

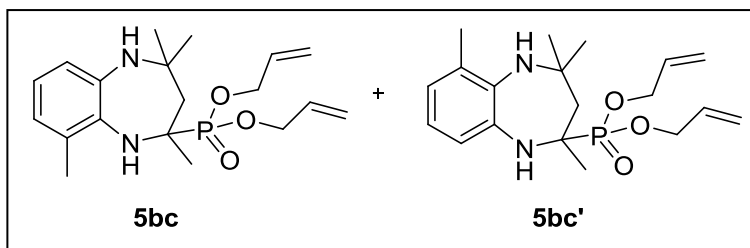


17H), 1.76-1.83 (m, 1H), 2.08-2.28 (m, 1H), 2.23 (s, 3H), 4.02-4.18 (m, 4H), 6.53-6.90 (m, 3H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ 13.6, 18.1, 18.7, 23.6, 30.5, 32.7 (d, <sup>3</sup>J<sub>PC</sub> = 5.5 Hz), 33.3, 43.4 (d, <sup>2</sup>J<sub>PC</sub> = 2.2 Hz), 52.8 (d, <sup>3</sup>J<sub>PC</sub> = 15.0 Hz), 56.3 (d, <sup>1</sup>J<sub>PC</sub> = 147.5 Hz), 66.5 (d, <sup>2</sup>J<sub>PC</sub> = 7.7 Hz), 66.6 (d, <sup>2</sup>J<sub>PC</sub> = 7.7 Hz), 120.5, 120.9, 124.2, 128.2, 136.1 (d, <sup>3</sup>J<sub>PC</sub> = 10.6 Hz), 137.1; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 29.94; MS (ESI): *m/z* 397.64 (M+H)<sup>+</sup>, 419.65 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>21</sub>H<sub>37</sub>N<sub>2</sub>O<sub>3</sub>P: C, 63.61; H, 9.41; N, 7.07. Found: C, 63.55; H, 9.50; N, 7.10.

**Diallyl 2,4,4,9-tetramethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5bc+5bc')** (1:5 regio-isomeric mixtures, only peaks corresponding to the major isomer are given).

Yield: 52%; yellow syrup; IR (CHCl<sub>3</sub>): 3355, 3019, 1599, 1520, 1466, 1384, 1319, 1215, 1029 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 1.28 (s, 3H), 1.32 (s, 3H), 1.63 (d,

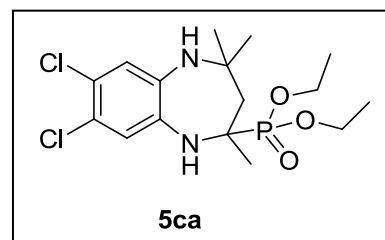
$^3J_{\text{PH}} = 17.6$  Hz, 3H), 1.75-1.85 (m, 1H), 2.12-2.19 (m, 1H), 2.23 (s, 3H), 4.55-4.66 (m, 4H), 5.15-5.44 (m, 4H), 5.86-



6.03 (m, 2H), 6.53-6.90 (m, 3H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  18.1, 23.6, 30.5, 33.3, 43.3, 52.8 (d,  $^3J_{\text{PC}} = 15.0$  Hz), 56.4 (d,  $^1J_{\text{PC}} = 147.1$  Hz), 67.1 (d,  $^2J_{\text{PC}} = 7.7$  Hz), 67.2 (d,  $^2J_{\text{PC}} = 7.7$  Hz), 118.0, 118.1, 120.6, 121.1, 124.3, 128.4, 132.9 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 133.0 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 135.9 (d,  $^3J_{\text{PC}} = 10.6$  Hz), 137.3;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  30.95; MS (ESI):  $m/z$  365.62 ( $\text{M}+\text{H}^+$ ), 387.62 ( $\text{M}+\text{Na}^+$ ); Anal. Calcd for  $\text{C}_{19}\text{H}_{29}\text{N}_2\text{O}_3\text{P}$ : C, 62.62; H, 8.02; N, 7.69. Found: C, 62.57; H, 8.09; N, 7.74.

**Diethyl 7,8-dichloro-2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5ca).**

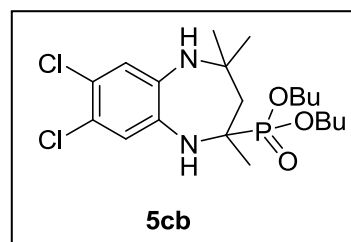
Yield: 59%; yellow syrup; IR ( $\text{CHCl}_3$ ): 3421, 3019, 1647, 1542, 1489, 1215, 1048  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.32-1.37 (m, 12H), 1.59 (d,  $^3J_{\text{PH}} = 16.8$  Hz, 3H), 1.77-1.82 (m, 1H), 2.17-2.24 (m, 1H), 4.13-4.25 (m, 4H), 6.72 (s, 1H), 6.79 (s, 1H);  $^{13}\text{C}$



NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.5 (d,  $^3J_{\text{PC}} = 6.6$  Hz), 16.6 (d,  $^3J_{\text{PC}} = 5.9$  Hz), 23.8, 30.8, 33.2, 43.3, 53.5 (d,  $^3J_{\text{PC}} = 13.9$  Hz), 56.2 (d,  $^1J_{\text{PC}} = 148.2$  Hz), 62.7 (d,  $^2J_{\text{PC}} = 8.1$  Hz), 63.2 (d,  $^2J_{\text{PC}} = 7.3$  Hz), 121.7, 122.1, 123.7, 123.9, 136.4 (d,  $^3J_{\text{PC}} = 11.0$  Hz), 137.1;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  29.08; MS (ESI):  $m/z$  395.12 ( $\text{M}+\text{H}^+$ ), 417.10 ( $\text{M}+\text{Na}^+$ ); Anal. Calcd for  $\text{C}_{16}\text{H}_{25}\text{Cl}_2\text{N}_2\text{O}_3\text{P}$ : C, 48.62; H, 6.38; N, 7.09. Found: C, 48.77; H, 6.44; N, 7.16.

**Dibutyl 7,8-dichloro-2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5cb).**

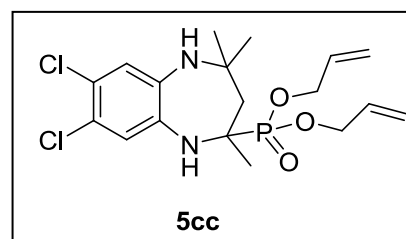
Yield: 55%; yellow syrup; IR ( $\text{CHCl}_3$ ): 3421, 3019, 2964, 1607, 1488, 1385, 1215, 1028  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.93 (2t,  $J = 7.3$  Hz, 6H), 1.29 (s, 3H), 1.33-1.42 (m, 4H), 1.37 (s, 3H), 1.58 (d,  $^3J_{\text{PH}} = 16.8$  Hz, 3H), 1.62-1.67 (m, 4H), 1.75-1.79 (m, 1H), 2.16-2.21 (m,



1H), 4.05-4.12 (m, 4H), 6.69 (s, 1H), 6.76 (s, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  13.6, 18.8, 24.1, 30.9, 32.6 (d,  $^3J_{\text{PC}} = 5.5$  Hz), 32.7 (d,  $^3J_{\text{PC}} = 5.5$  Hz), 33.2, 43.2, 53.5 (d,  $^3J_{\text{PC}} = 13.6$  Hz), 56.5 (d,  $^1J_{\text{PC}} = 148.1$  Hz), 66.5 (d,  $^2J_{\text{PC}} = 8.2$  Hz), 66.8 (d,  $^3J_{\text{PC}} = 7.3$  Hz), 121.5, 122.1, 123.6, 123.9, 136.3 (d,  $^3J_{\text{PC}} = 9.9$  Hz), 137.0;  $^{31}\text{P}$  NMR (202 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.91; MS (ESI):  $m/z$  383.32 (M+H) $^+$ , 405.31 (M+Na) $^+$ ; Anal. Calcd for  $\text{C}_{20}\text{H}_{33}\text{Cl}_2\text{N}_2\text{O}_3\text{P}$ : C, 53.22; H, 7.37; N, 6.21. Found: C, 53.25, H, 7.43, N, 6.24.

**Diallyl 7,8-dichloro-2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5cc).**

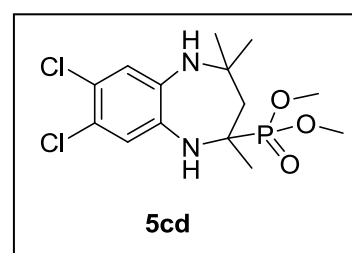
Yield: 52%; yellow syrup; IR ( $\text{CHCl}_3$ ): 3421, 3019, 1611, 1423, 1215, 1019  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.29 (s, 3H), 1.36 (s, 3H), 1.61 (d,  $^3J_{\text{PH}} = 17.2$  Hz, 3H), 1.74-1.84 (m, 1H), 2.16-2.35 (m, 1H), 4.53-4.62 (m, 4H), 5.23-5.38 (m, 4H), 5.83-6.02 (m,



2H), 6.69 (s, 1H), 6.77 (s, 1H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  24.1, 30.9, 33.2, 43.2 (d,  $^2J_{\text{PC}} = 1.1$  Hz), 53.5 (d,  $^3J_{\text{PC}} = 13.9$  Hz), 56.5 (d,  $^1J_{\text{PC}} = 147.1$  Hz), 67.1 (d,  $^2J_{\text{PC}} = 7.7$  Hz), 67.4 (d,  $^2J_{\text{PC}} = 7.0$  Hz), 118.3, 118.4, 121.6, 122.2, 123.5, 124.1, 132.8, 132.9, 135.6 (d,  $^2J_{\text{PC}} = 10.6$  Hz), 137.1;  $^{31}\text{P}$  NMR (161 MHz,  $\text{CDCl}_3$ ):  $\delta$  29.68; MS (ESI):  $m/z$  420.31 (M+H) $^+$ ; Anal. Calcd for  $\text{C}_{18}\text{H}_{25}\text{Cl}_2\text{N}_2\text{O}_3\text{P}$ : C, 51.56; H, 6.01; N, 6.68. Found: C, 51.64, H, 6.15, N, 6.74.

**Dimethyl 7,8-dichloro-2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5cd).**

Yield: 51%; yellow syrup; IR ( $\text{CHCl}_3$ ): 3421, 3019, 2964, 1622, 1542, 1488, 1385, 1216, 1031  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.30 (s, 3H), 1.34 (s, 3H), 1.58 (d,  $^3J_{\text{PH}} = 17.2$  Hz, 3H), 1.73-1.83 (m, 1H), 2.05-2.25 (m, 1H), 3.79 (d,  $^3J_{\text{PH}} = 10.4$ , 3H), 3.82 (d,  $^3J_{\text{PH}} =$



10.3, 3H), 6.72 (s, 1H), 6.81 (s, 1H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  23.9, 30.7, 33.2, 43.4, 53.4 (d,  $^2J_{\text{PC}} = 7.7$  Hz), 53.5 (d,  $^3J_{\text{PC}} = 13.9$  Hz), 54.0 (d,  $^2J_{\text{PC}} = 7.0$  Hz), 56.1 (d,  $^1J_{\text{PC}} = 148.6$  Hz), 121.8, 122.2, 123.7, 124.1, 136.2 (d,  $^3J_{\text{PC}} = 10.9$  Hz), 137.1;  $^{31}\text{P}$  NMR (202 MHz,  $\text{CDCl}_3$ ):  $\delta$  31.33; MS (ESI):  $m/z$  367.17 (M+H) $^+$ , 389.16 (M+Na) $^+$ ;

Anal. Calcd for  $C_{14}H_{21}Cl_2N_2O_3P$ : C, 45.79; H, 5.76; N, 7.63. Found: C, 45.83; H, 5.82, N, 7.66.

**Diethyl 2,4,4-trimethyl-8-nitro-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5da+5da')** (1:8 regio-isomeric mixtures, only peaks corresponding to the major isomer are given)

Yield: 63%; yellow syrup;

IR ( $CHCl_3$ ): 3421, 3367,

3019, 1614, 1519, 1216,

1054  $cm^{-1}$ ;  $^1H$  NMR (200

MHz,  $CDCl_3$ ):  $\delta$  1.26 (t,  $J$

= 7.0 Hz, 6H), 1.39 (s, 3H), 1.59 (d,  $^3J_{PH} = 16.7$  Hz, 3H), 1.57 (s, 3H), 1.76-1.85 (m, 1H), 2.37-2.53 (m, 1H), 4.01-4.21 (m, 4H), 6.63 (d,  $J = 8.7$  Hz, 1H), 7.58-7.90 (m, 2H);

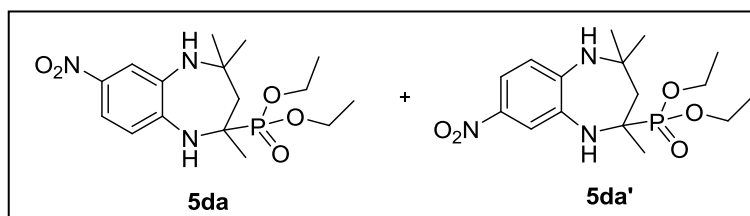
$^{13}C$  NMR (50 MHz,  $CDCl_3$ ):  $\delta$  16.4 (d,  $^3J_{PC} = 5.5$  Hz), 16.5 (d,  $^3J_{PC} = 5.5$  Hz), 25.0,

32.0, 33.5, 43.1, 54.3 (d,  $^1J_{PC} = 11.7$  Hz), 55.2 (d,  $^1J_{PC} = 145.6$  Hz), 62.5 (d,  $^2J_{PC} = 8.0$

Hz), 62.7 (d,  $^2J_{PC} = 7.3$  Hz), 116.5, 117.4, 119.3, 132.3 (d,  $^3J_{PC} = 7.0$  Hz), 138.9,

145.3;  $^{31}P$  NMR (161 MHz,  $CDCl_3$ ):  $\delta$  28.35; MS (ESI):  $m/z$  372.67 ( $M+H$ ) $^+$ , 394.73

( $M+Na$ ) $^+$ ; Anal. Calcd for  $C_{16}H_{26}N_3O_5P$ : C, 51.75; H, 7.06; N, 11.31. Found: 51.81; H, 7.17, N, 11.23



**Dibutyl 2,4,4-trimethyl-8-nitro-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5db).**

Yield: 59%; yellow syrup; IR ( $CHCl_3$ ): 3420, 3367,

3019, 2964, 1593, 1518, 1319, 1216, 1024  $cm^{-1}$ ;  $^1H$

NMR (400 MHz,  $CDCl_3$ ):  $\delta$  0.87-0.95 (m, 6H), 1.27-

1.61 (m, 8H), 1.38 (s, 3H), 1.57 (s, 3H), 1.60 (d,  $^3J_{PH}$

= 16.5 Hz, 3H), 1.78-1.83 (m, 1H), 2.40-2.48 (m, 1H),

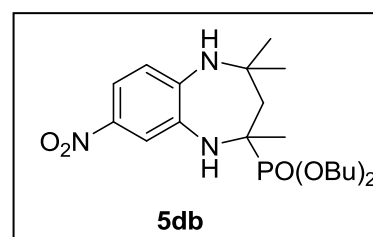
3.74 (bs, 1H), 3.94-4.11 (m, 4H), 4.18 (bs, 1H), 6.40 (d,  $J = 8.7$  Hz, 1H), 7.56 (d,  $J =$

2.4 Hz, 1H), 7.66 (dd,  $J = 8.7, 2.4$  Hz, 1H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  13.6, 18.7,

18.8, 25.1 (d,  $^4J_{PC} = 1.1$  Hz), 32.1, 32.6 (d,  $^3J_{PC} = 3.3$  Hz), 32.7 (d,  $^3J_{PC} = 3.3$  Hz),

33.6, 43.0, 54.3 (d,  $^3J_{PC} = 11.7$  Hz), 56.9 (d,  $^1J_{PC} = 145.3$  Hz), 66.3 (d,  $^2J_{PC} = 7.7$  Hz),

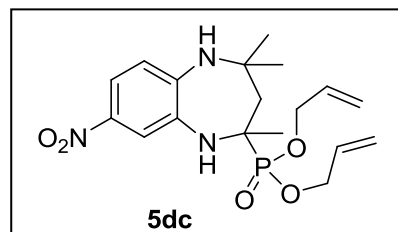
66.5 (d,  $^2J_{PC} = 7.7$  Hz), 116.5, 117.5, 119.3, 132.3 (d,  $^3J_{PC} = 6.7$  Hz), 139.2, 145.1;  $^{31}P$



NMR (161 MHz, CDCl<sub>3</sub>):  $\delta$  25.7; MS (ESI):  $m/z$  428.2 (M+H)<sup>+</sup>, 450.2 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>20</sub>H<sub>34</sub>N<sub>3</sub>O<sub>5</sub>P: C, 56.19; H, 8.02; N, 9.83. Found: C, 56.10; H, 8.14; N, 9.96

**Diallyl 2,4,4-trimethyl-8-nitro-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5dc).**

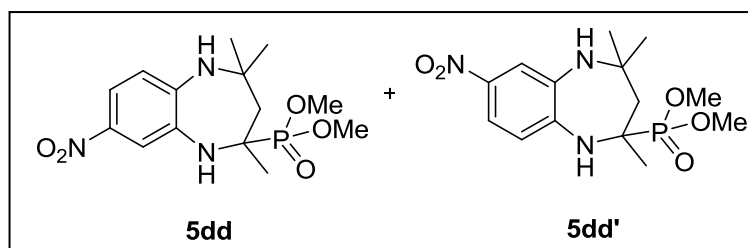
Yield: 57%; yellow syrup; IR (CHCl<sub>3</sub>): 3421, 3367, 3020, 1593, 1519, 1484, 1320, 1215, 1030 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  1.38 (s, 3H), 1.59 (s, 3H), 1.62 (d, <sup>3</sup>J<sub>PH</sub> = 16.6 Hz, 3H), 1.80-1.84 (m, 1H), 2.47-



2.53 (m, 1H), 4.38-4.58 (m, 4H), 5.20-5.30 (m, 4H), 5.80-5.88 (m, 2H), 6.41 (d,  $J$  = 8.7 Hz, 1H), 7.58 (d,  $J$  = 2.5 Hz, 1H), 7.66 (dd,  $J$  = 8.7, 2.5 Hz, 1H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  25.2, 32.0, 33.6, 43.0, 54.2, 57.0 (d, <sup>1</sup>J<sub>PC</sub> = 143.5 Hz), 66.8 (d, <sup>2</sup>J<sub>PC</sub> = 7.3 Hz), 67.0 (d, <sup>2</sup>J<sub>PC</sub> = 7.3 Hz), 116.4, 117.5, 118.2, 118.4, 119.4, 132.1 (d, <sup>3</sup>J<sub>PC</sub> = 6.3 Hz), 132.7 (d, <sup>3</sup>J<sub>PC</sub> = 5.3 Hz), 132.8 (d, <sup>3</sup>J<sub>PC</sub> = 5.3 Hz), 139.0, 145.3; <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>):  $\delta$  29.06; MS (ESI):  $m/z$  396.48 (M+H)<sup>+</sup>, 418.43 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>18</sub>H<sub>26</sub>N<sub>3</sub>O<sub>5</sub>P: C, 54.68; H, 6.63; N, 10.63. Found: C, 54.75, 6.53. N, 10.59.

**Dimethyl 2,4,4-trimethyl-8-nitro-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5dd+5dd')** (1:1 regio-isomeric mixtures, only peaks corresponding to the major isomer are given).

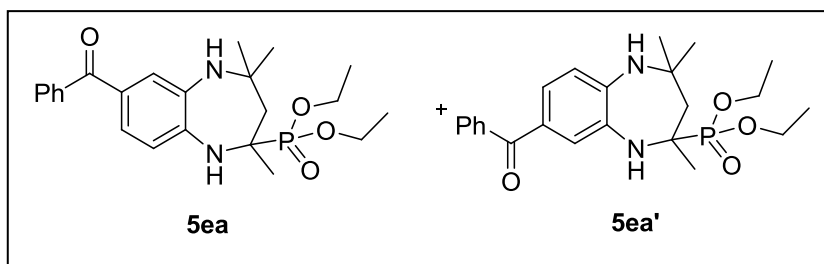
Yield: 53%; yellow syrup; IR (CHCl<sub>3</sub>): 3420, 3360, 3019, 2923, 1592, 1518, 1318, 1215, 1046 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):



$\delta$  1.28 (s, 3H), 1.38 (s, 3H), 1.70 (d,  $J$  = 16.6 Hz, 3H), 1.79-1.87 (m, 1H), 2.15-2.45 (m, 1H), 3.68 (d,  $J$  = 10.3 Hz, 3H), 3.79 (d,  $J$  = 10.3 Hz, 3H), 6.42 (d,  $J$  = 8.8 Hz, 1H), 7.53-7.64 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  25.1, 31.2, 32.4, 43.2, 53.6 (d, <sup>2</sup>J<sub>PC</sub> 8.1 Hz), 54.1 (d, <sup>2</sup>J<sub>PC</sub> = 7.3 Hz), 53.7 (d, <sup>3</sup>J<sub>PC</sub> = 11.0 Hz), 57.0 (d, <sup>1</sup>J<sub>PC</sub> = 149.7 Hz), 116.4, 118.2, 119.4, 135.1, 140.9, 145.2; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>):  $\delta$  30.80; MS (ESI):  $m/z$  344.28 (M+H)<sup>+</sup>, 366.25 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>14</sub>H<sub>22</sub>N<sub>3</sub>O<sub>5</sub>P: C, 49.98; H, 6.46; N, 12.24. Found: C, 49.87; H, 6.52; N, 12.22.

**Diethyl 7-benzoyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-yl-phosphonate (5ea+5ea')** (3:4 regio-isomeric mixtures, only peaks corresponding to the major isomer are given).

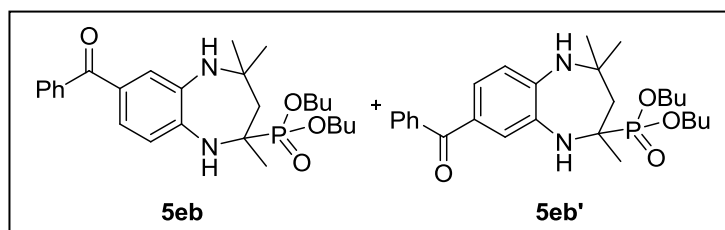
Yield: 43%; yellow syrup; IR (CHCl<sub>3</sub>): 3420, 3368, 3019, 1730, 1620, 1515, 1446, 1319, 1215,



cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 1.24-1.48 (m, 12H), 1.58 (d, <sup>3</sup>J<sub>PH</sub> = 16.9 Hz, 3H), 1.77-1.89 (m, 1H), 2.14-2.46 (m 1H), 4.05-4.24 (m, 4H), 6.52-6.68 (d, *J* = 8.7 Hz, 1H), 7.21-7.28 (m, 2H), 7.40-7.57 (m, 3H), 7.69-7.74 (m, 2H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ 16.4 (d, <sup>3</sup>J<sub>PC</sub> = 4.4 Hz), 16.6 (d, <sup>3</sup>J<sub>PC</sub> = 4.4 Hz), 24.6, 31.9, 33.5, 43.5, 54.0 (d, <sup>3</sup>J<sub>PC</sub> = 13.5 Hz), 56.5 (d, <sup>1</sup>J<sub>PC</sub> = 146.7 Hz), 62.9 (d, <sup>2</sup>J<sub>PC</sub> = 7.0 Hz), 63.4 (d, <sup>2</sup>J<sub>PC</sub> = 7.0 Hz), 117.9, 123.9, 126.1, 128.1, 130.0, 129.5, 131.4, 135.5, 138.9, 143.0, 195.2; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 29.08; MS (ESI): *m/z* 431.76 (M+H)<sup>+</sup>, 453.77 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>23</sub>H<sub>31</sub>N<sub>2</sub>O<sub>4</sub>P: C, 64.17; H, 7.26; N, 6.51. Found: C, 64.27; H, 7.30, N, 6.50

**Dibutyl 7-benzoyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5eb+5eb')** (1:3 regio-isomeric mixtures, only peaks corresponding to the major isomer are given).

Yield: 42%; yellow syrup; IR (CHCl<sub>3</sub>): 3421, 3392, 3018, 1641, 1593, 1480, 1320, 1216 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 0.90



(t, *J* = 7.2, 6H), 1.19 (m, 17H), 1.82-2.41 (m, 2H), 4.00-4.15 (m, 4H), 6.52 (d, *J* = 8.6 Hz, 1H), 7.23-7.30 (m, 2H), 7.40-7.56 (m, 3H), 7.69-7.74 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 13.6, 18.7, 24.7, 31.2, 33.4, 32.6 (d, <sup>3</sup>J<sub>PC</sub> = 5.8 Hz), 32.6 (d, <sup>3</sup>J<sub>PC</sub> = 5.5 Hz), 43.5, 53.9 (d, <sup>3</sup>J<sub>PC</sub> = 13.5 Hz), 56.8 (d, <sup>1</sup>J<sub>PC</sub> = 146.4 Hz), 66.3 (d, <sup>2</sup>J<sub>PC</sub> = 8.0 Hz), 66.6 (d, <sup>2</sup>J<sub>PC</sub> = 7.7 Hz), 119.2, 123.9, 126.1, 128.1, 128.5, 129.5, 131.3, 135.4, 138.9, 143.0, 195.5; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 28.96; MS (ESI): *m/z* 487.94 (M+H)<sup>+</sup>, 509.95 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>27</sub>H<sub>39</sub>N<sub>2</sub>O<sub>4</sub>P: C, 66.65; H, 8.08; N, 5.76. Found: C, 66.70, H, 8.13, N, 5.68.

**Diallyl 7-benzoyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5ec+5ec')** (1:2 regio-isomeric mixture, only peaks corresponding to the major isomer are given).

Yield: 50%;

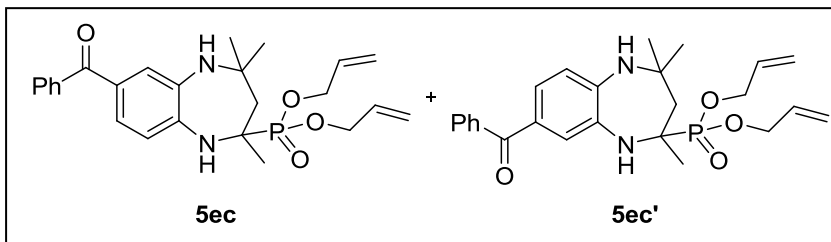
yellow syrup; IR

(CHCl<sub>3</sub>): 3370,

3420, 3019, 1641,

1521, 1476, 1422,

1215 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ 1.38 (s, 3H), 1.50 (s, 3H), 1.61 (d, <sup>3</sup>J<sub>PH</sub> = 17.1 Hz, 3H), 1.79-1.88 (m, 1H), 2.35-2.51 (m, 1H), 4.43-4.63 (m, 4H), 5.14-5.49 (m, 4H), 5.78-5.97 (m, 2H), 6.51 (d, *J* = 8.6 Hz, 1H), 7.23-7.29 (m, 1H), 7.39-7.54 (m, 1H), 7.67-7.78 (m, 2H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): δ 24.8, 31.9, 33.5, 43.4, 54.0 (d, <sup>3</sup>J<sub>PC</sub> = 12.4 Hz), 56.9 (d, <sup>1</sup>J<sub>PC</sub> = 144.9 Hz), 66.9 (d, <sup>2</sup>J<sub>PC</sub> = 8.0 Hz), 67.2 (d, <sup>2</sup>J<sub>PC</sub> = 7.0 Hz), 117.8, 118.1, 118.2, 124.1, 128.2, 128.1, 128.7, 129.5, 131.4, 132.9, 133.2, 138.9, 142.9, 195.1; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 29.79; MS (ESI): *m/z* 455.44 (M+H)<sup>+</sup>, 477.29 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>25</sub>H<sub>31</sub>N<sub>2</sub>O<sub>4</sub>P: C, 66.07; H, 6.87; N, 6.16. Found: C, 66.13; H, 6.84; N, 6.21.



**Dimethyl 7-benzoyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-2-ylphosphonate (5ed+5ed')** (1:1 regio-isomeric mixture, only peaks corresponding to the major isomer are given).

Yield: 49%; yellow syrup;

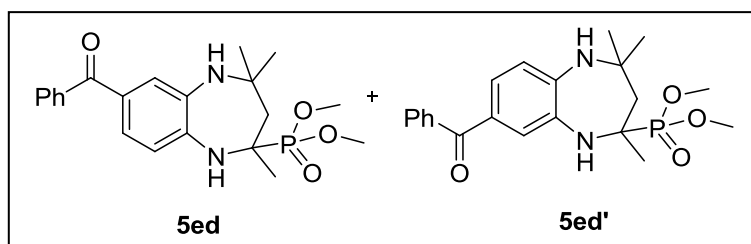
IR (CHCl<sub>3</sub>): 3420, 3018,

2956, 1653, 1641, 1593,

1508, 1338, 1217, 1132,

1053 cm<sup>-1</sup>; <sup>1</sup>H NMR (400

MHz, CDCl<sub>3</sub>): δ 1.37 (s, 3H), 1.39 (s, 3H), 1.70 (d, <sup>3</sup>J<sub>PH</sub> = 16.6 Hz, 3H), 1.78-1.88 (m, 1H), 2.20-2.40 (m, 1H), 3.74 (m, 6H), 6.69 (d, *J* = 8.6 Hz, 1H), 7.23-7.27 (m, 2H), 7.43-7.54 (m, 3H), 7.72-7.82 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 24.6, 30.9, 32.7, 43.1, 53.2 (d, <sup>2</sup>J<sub>PC</sub> = 7.3 Hz), 53.4, 56.9 (d, <sup>1</sup>J<sub>PC</sub> = 149.6 Hz), 118.2, 123.9, 125.8, 128.1, 129.6, 130.3, 131.6, 133.4, 138.7, 141.9, 195.2; <sup>31</sup>P NMR (161 MHz, CDCl<sub>3</sub>): δ 31.5; MS (ESI): *m/z* 403.62 (M+H)<sup>+</sup>, 425.69 (M+Na)<sup>+</sup>; Anal. Calcd for C<sub>21</sub>H<sub>27</sub>N<sub>2</sub>O<sub>4</sub>P: C, 62.68; H, 6.76; N, 6.96. Found: C, 62.75; H, 6.72; N, 6.91.



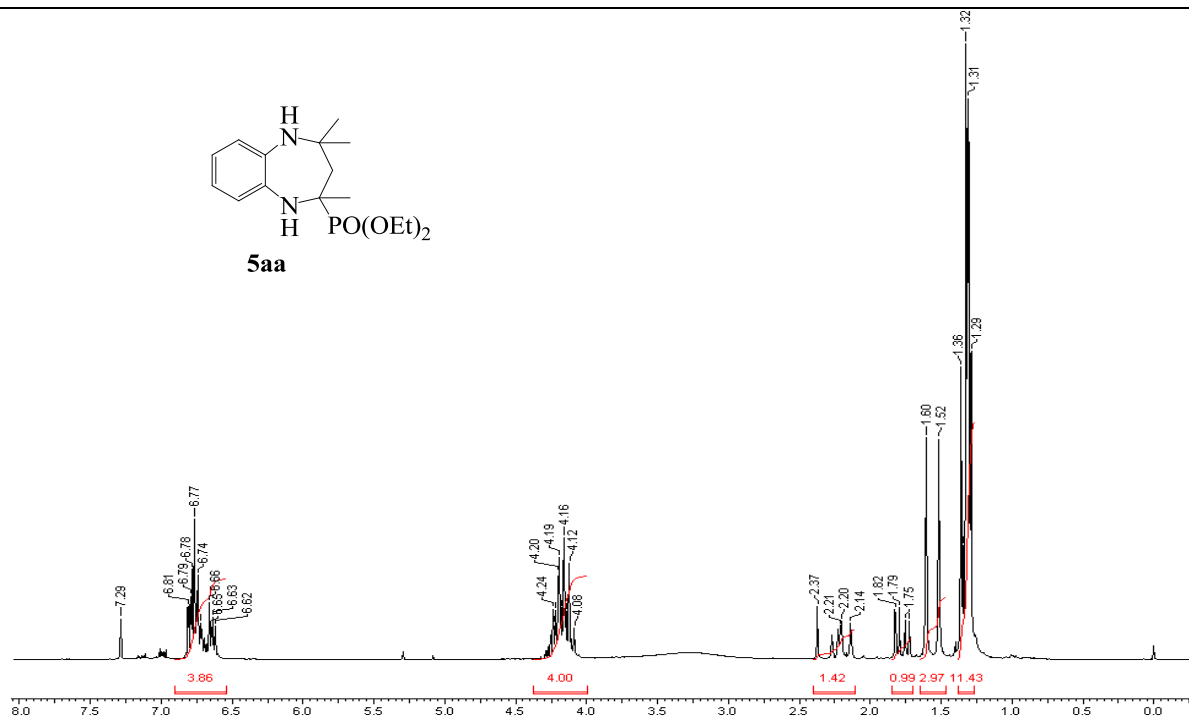
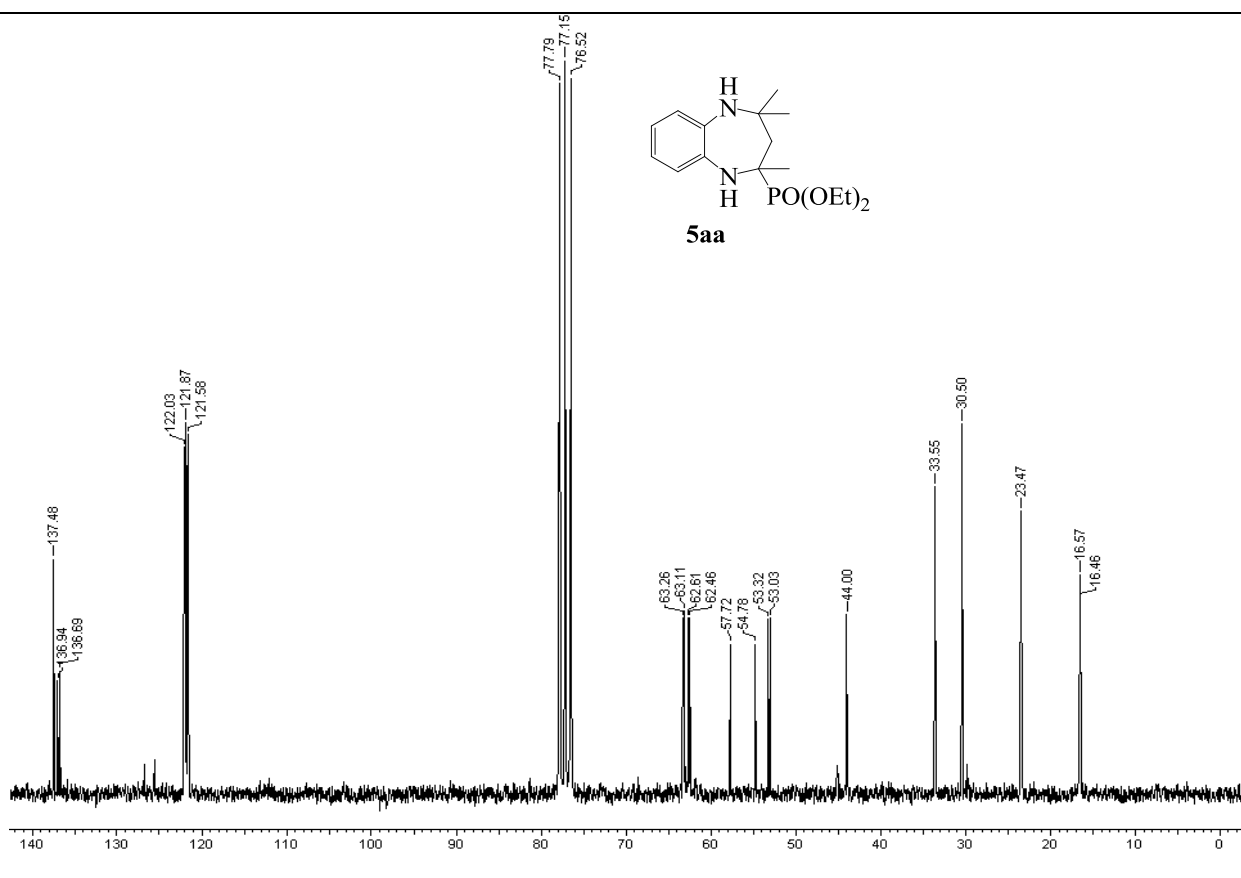


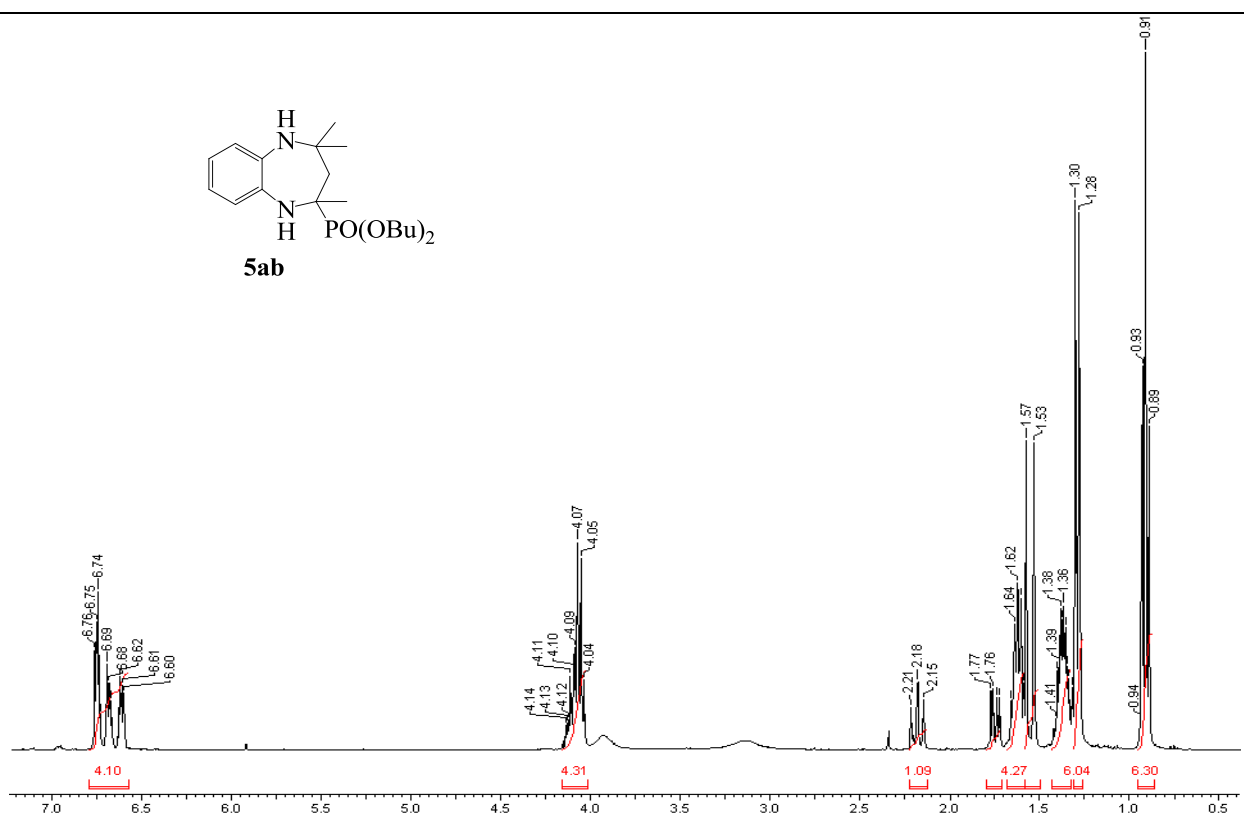
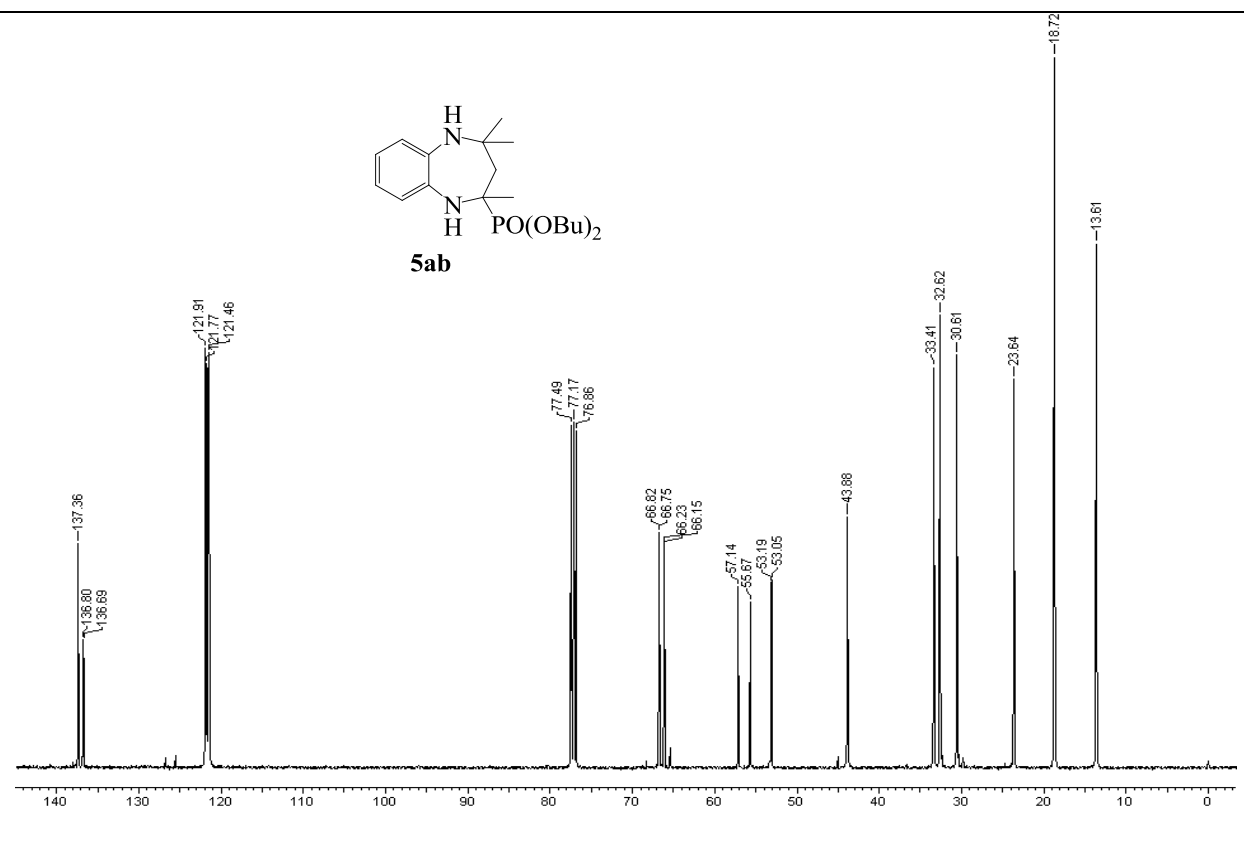
## Biology

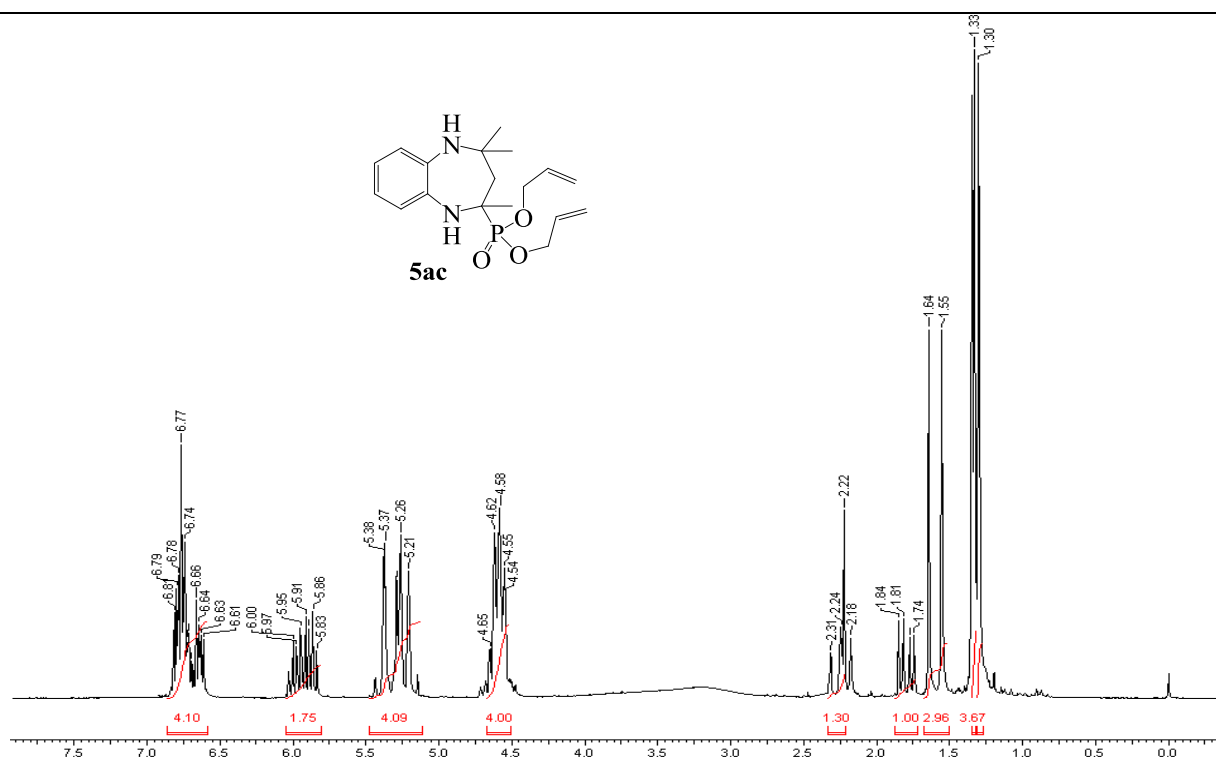
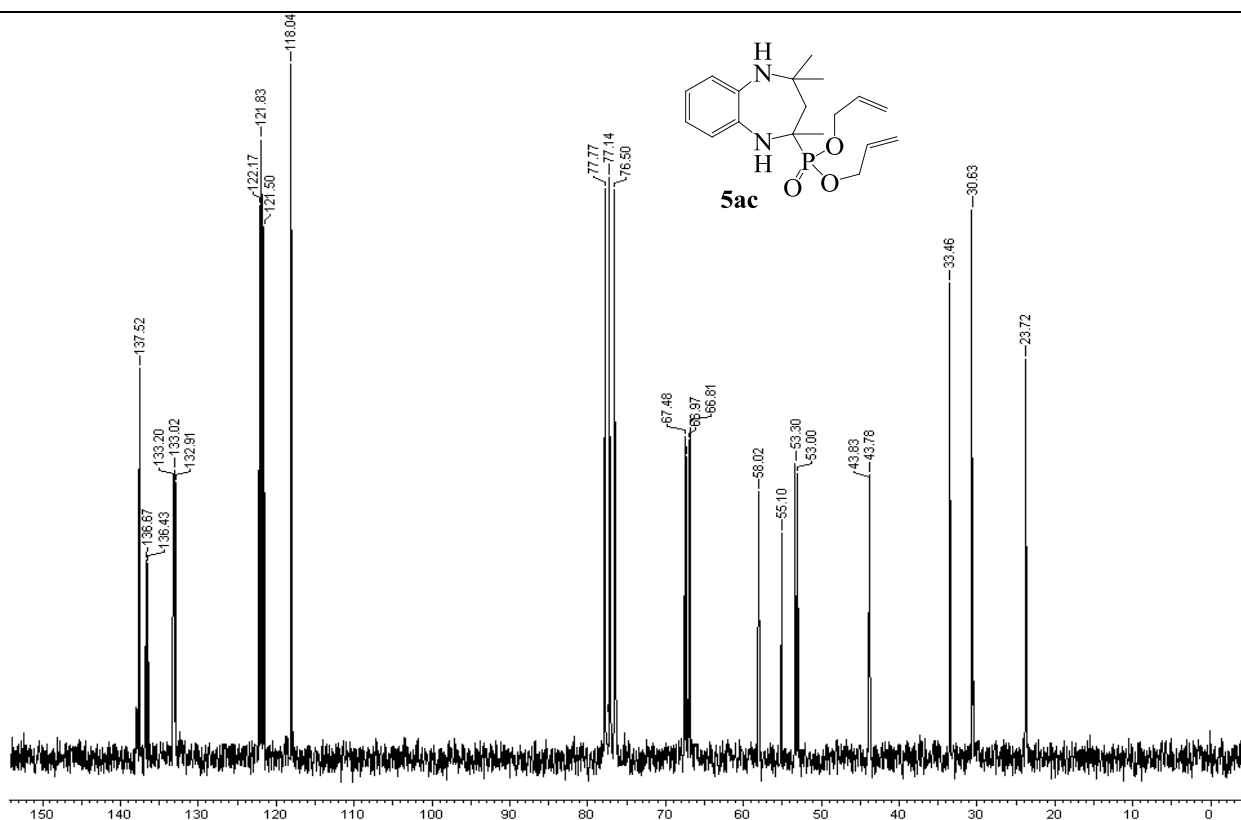
### Bioassay of synthesized BDPs

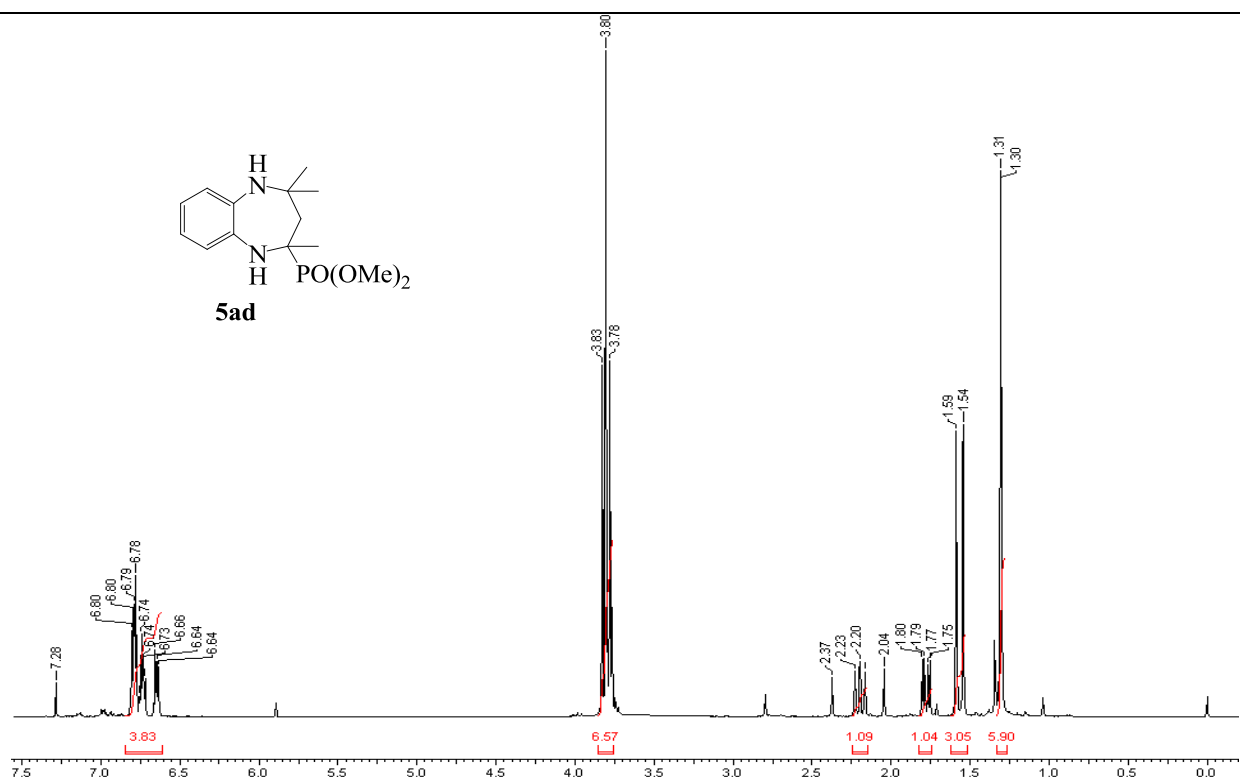
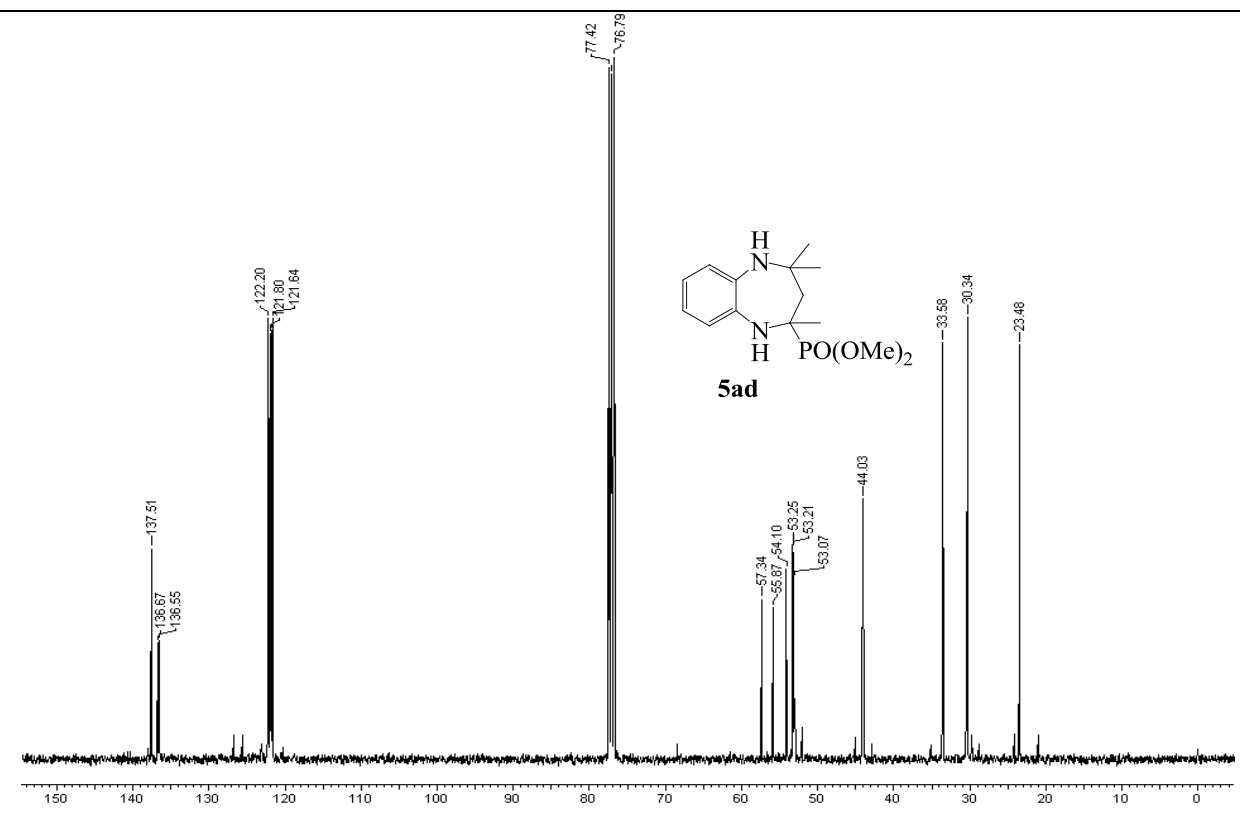
The inhibitory activity of cysteine protease inhibitor (CPI) against clostripain was assayed spectrophotometrically.<sup>21</sup> Clostripain was activated in 10 mM Tris HCL buffer, pH 7.4, containing 1 mM CaCl<sub>2</sub> and 2.5 mM DTT for 3 h at 37 °C. After activation, clostripain (25 nM) was added to enzyme buffer (100 mM Tris HCl buffer, pH 7.4) containing the substrate BAPNA (500 μM) in the presence and absence of CPI. Formation of product (*p*-nitroaniline) was monitored by the increase in absorbance at 410 nm.

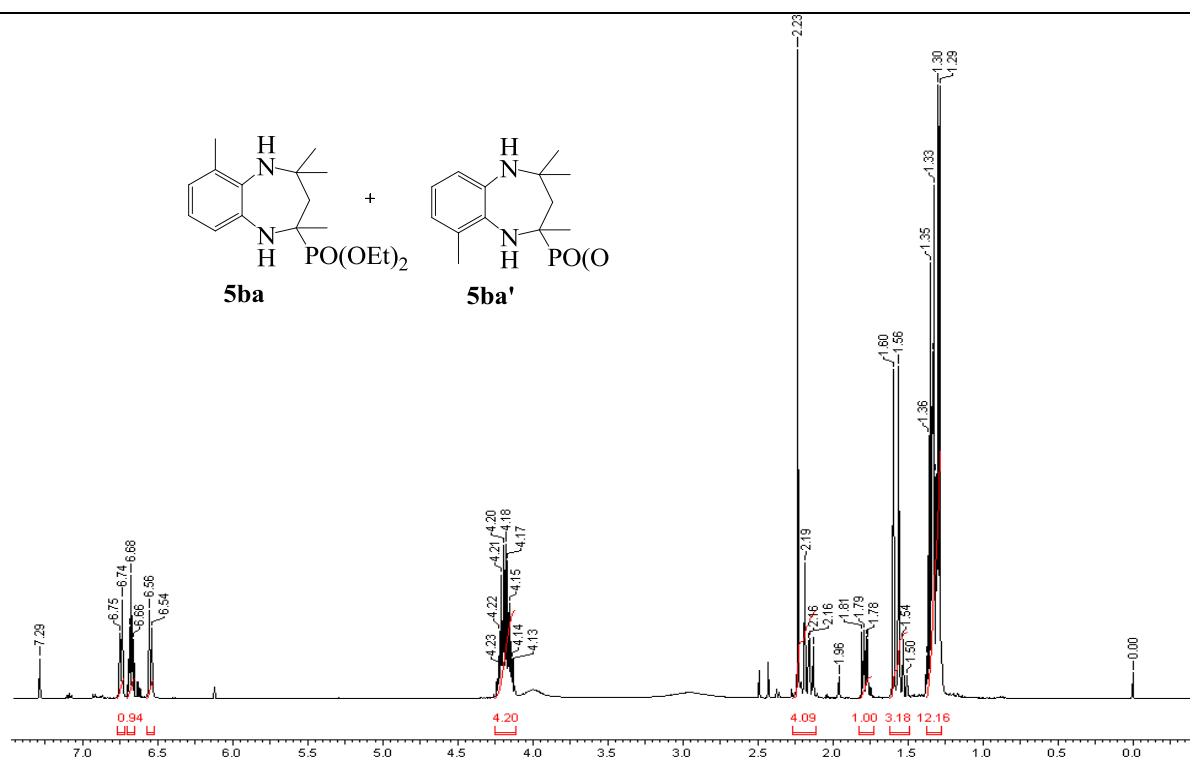
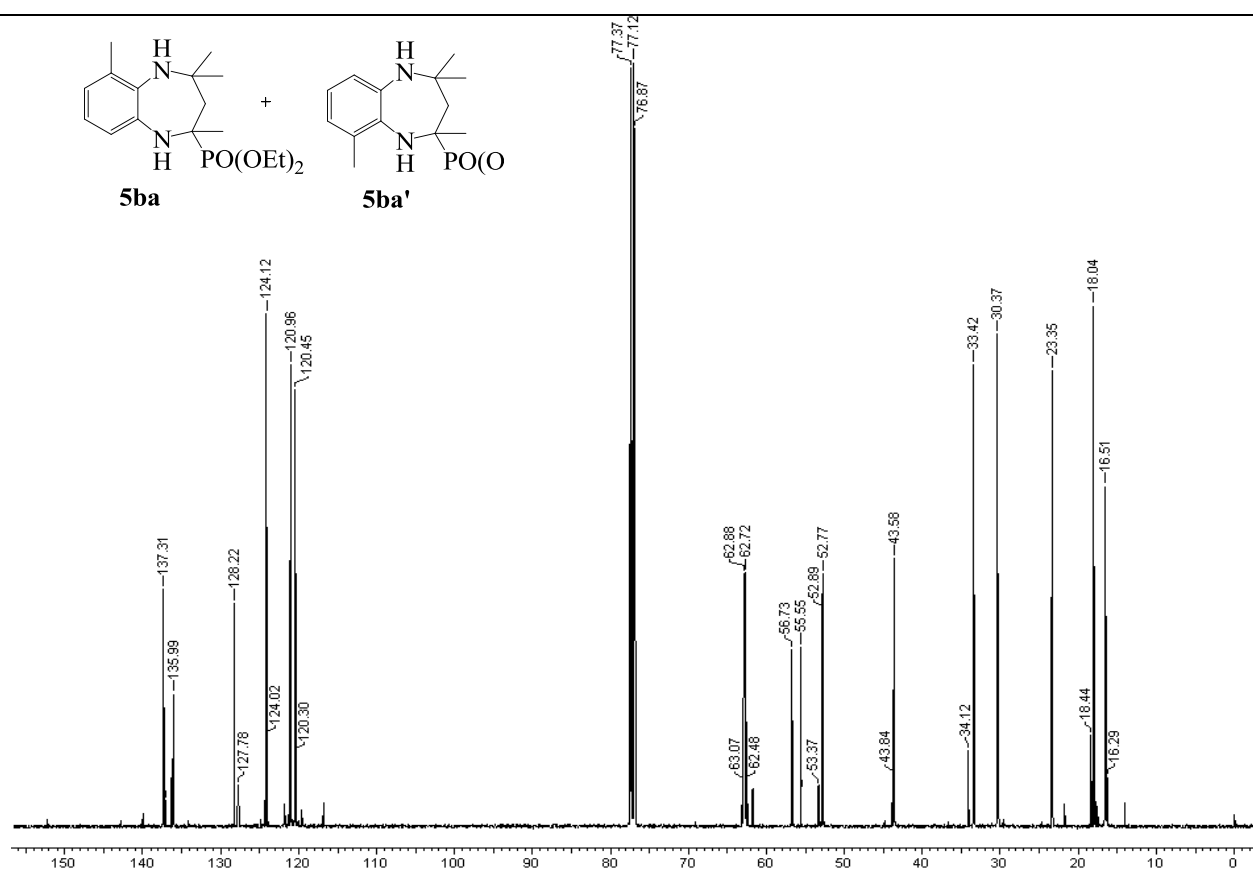
## Spectra

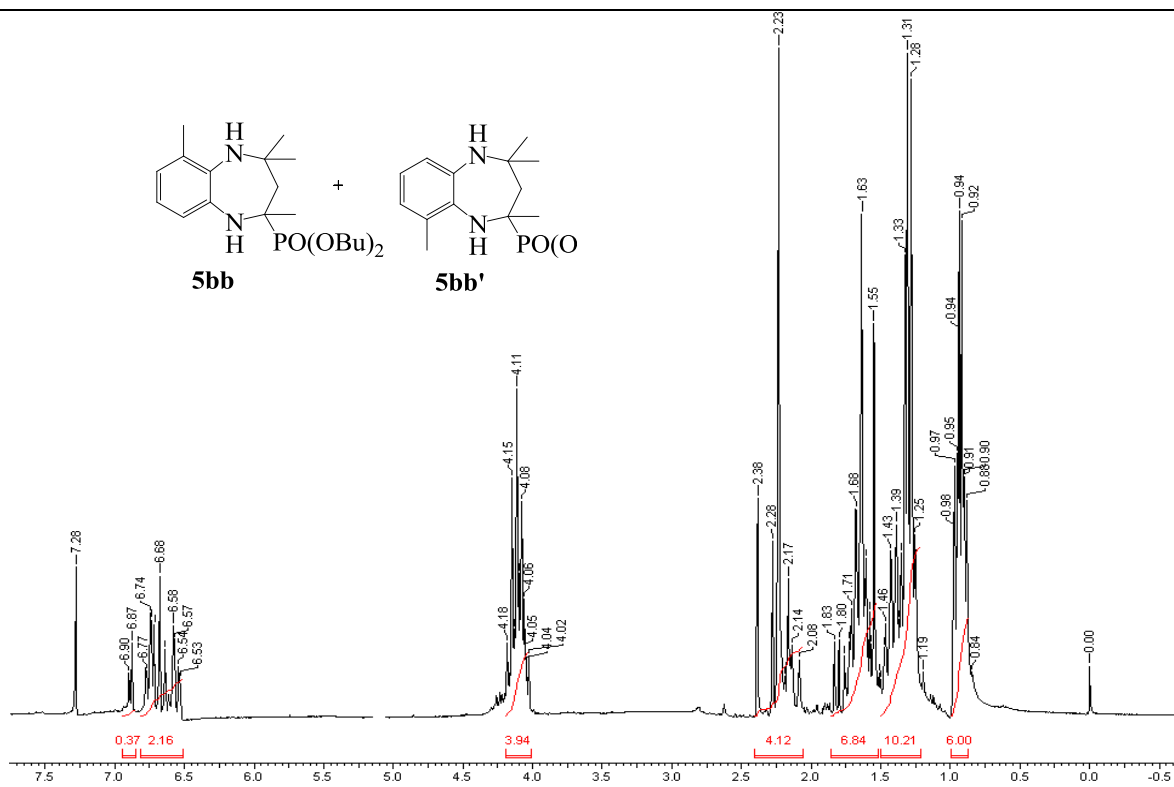
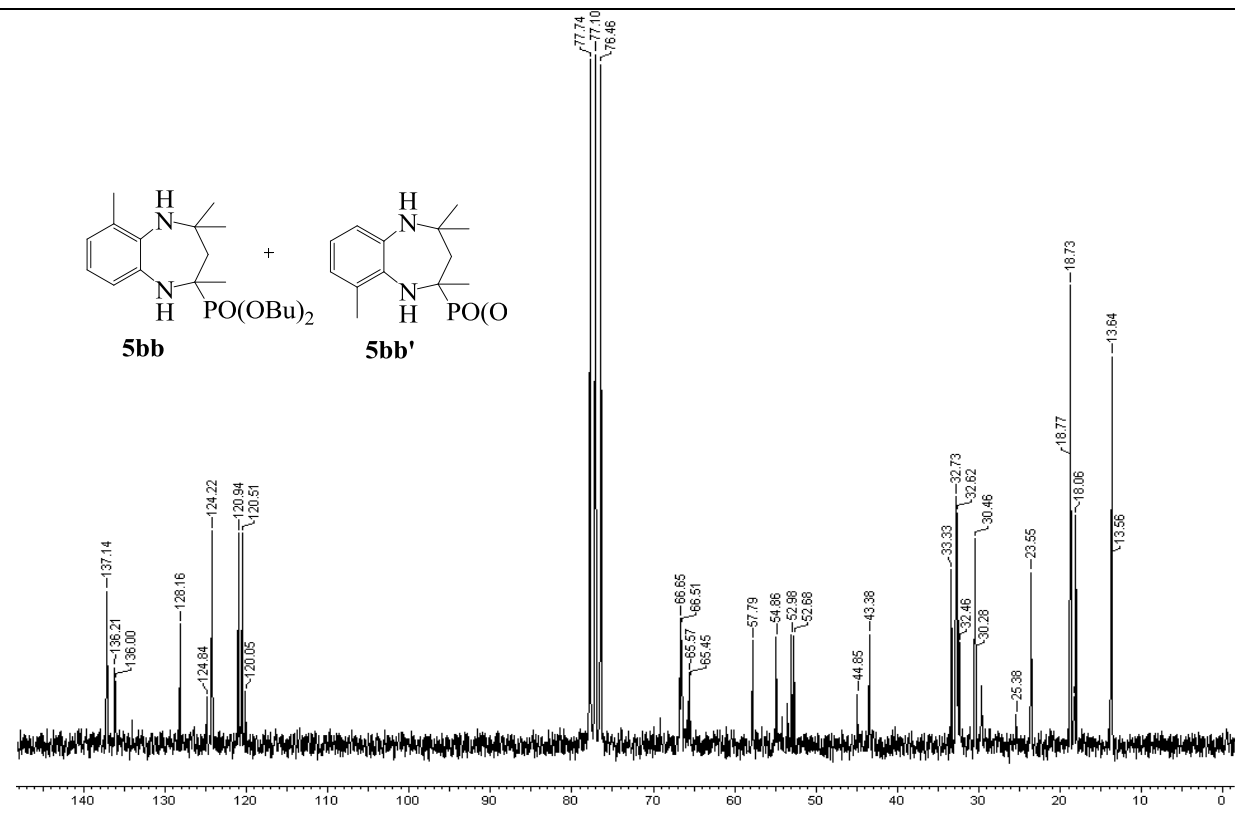
 $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **5aa** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **5aa**

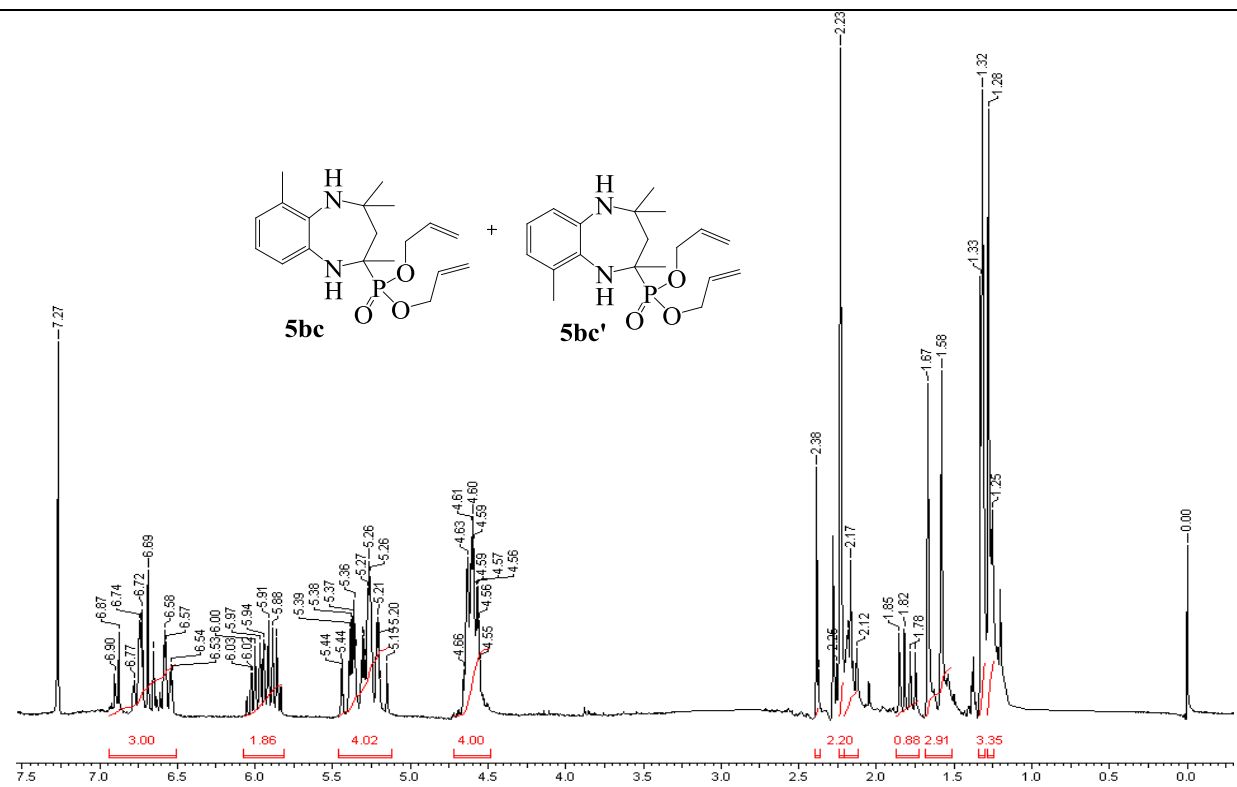
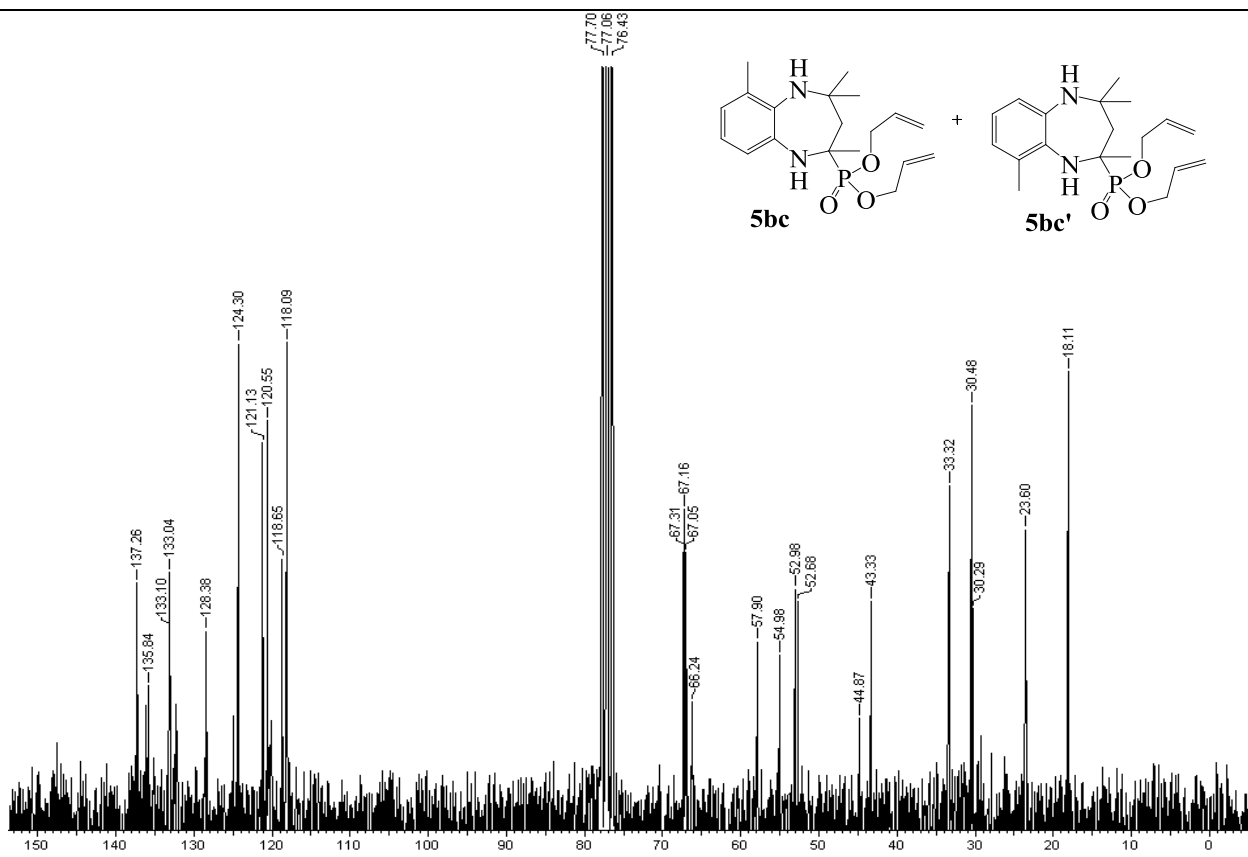
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) of **5ab** $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) of **5ab**

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **5ac** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **5ac**

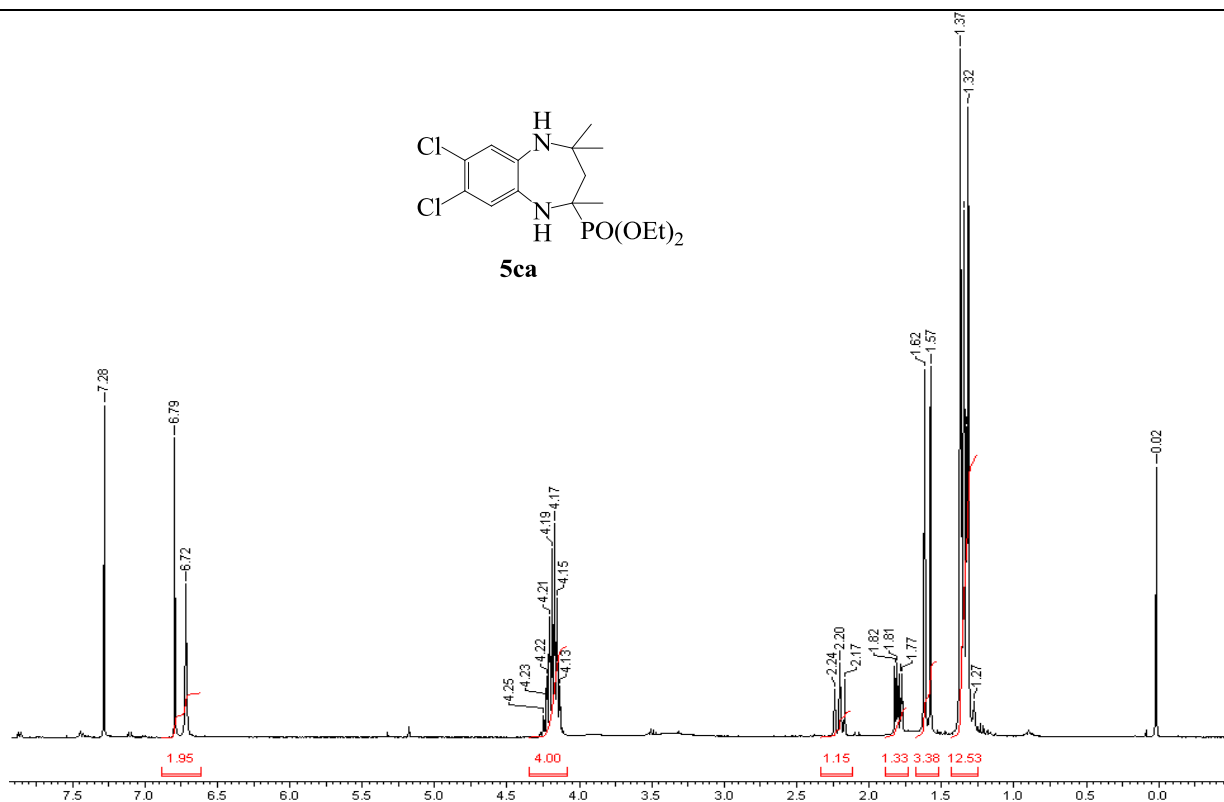
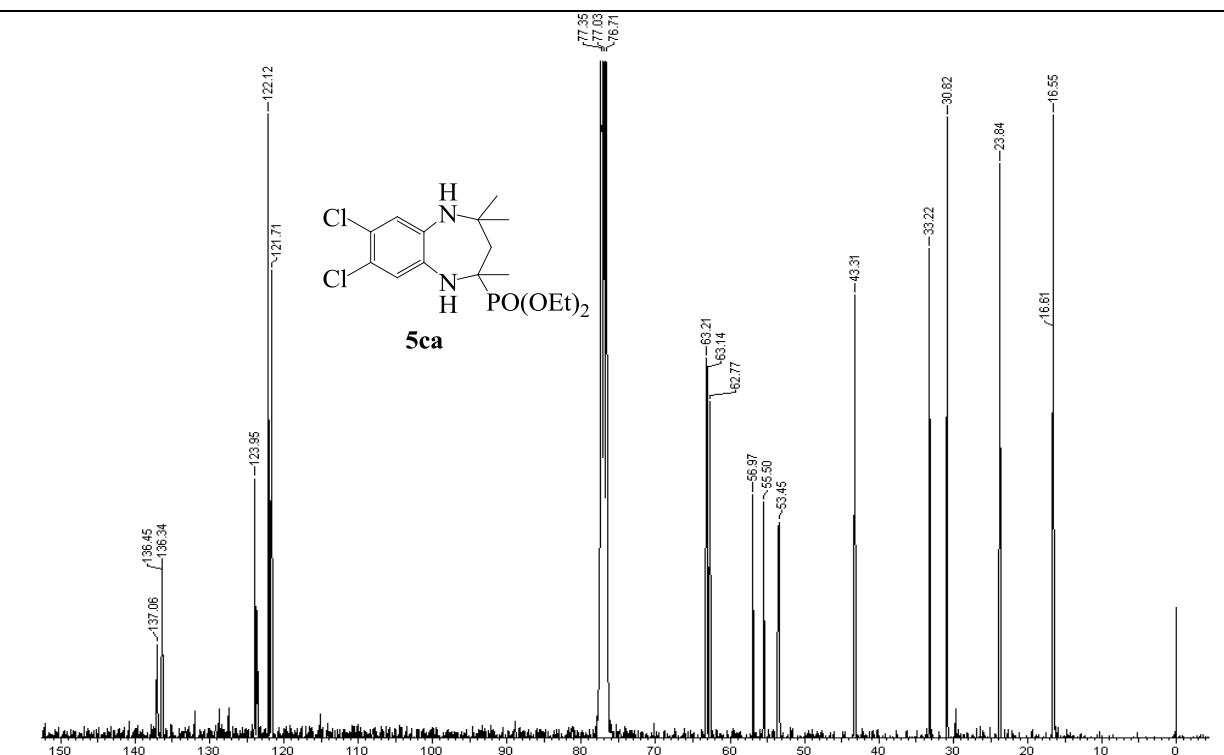
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) of **5ad** $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) of **5ad**

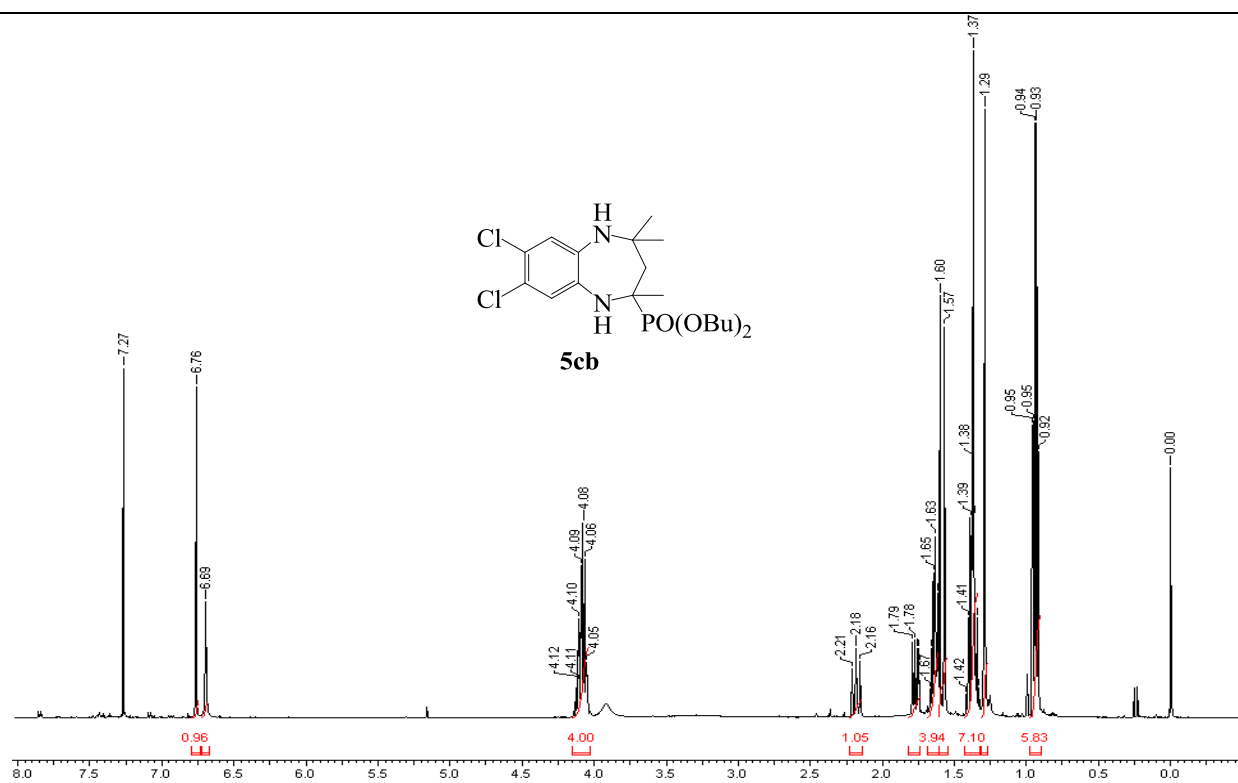
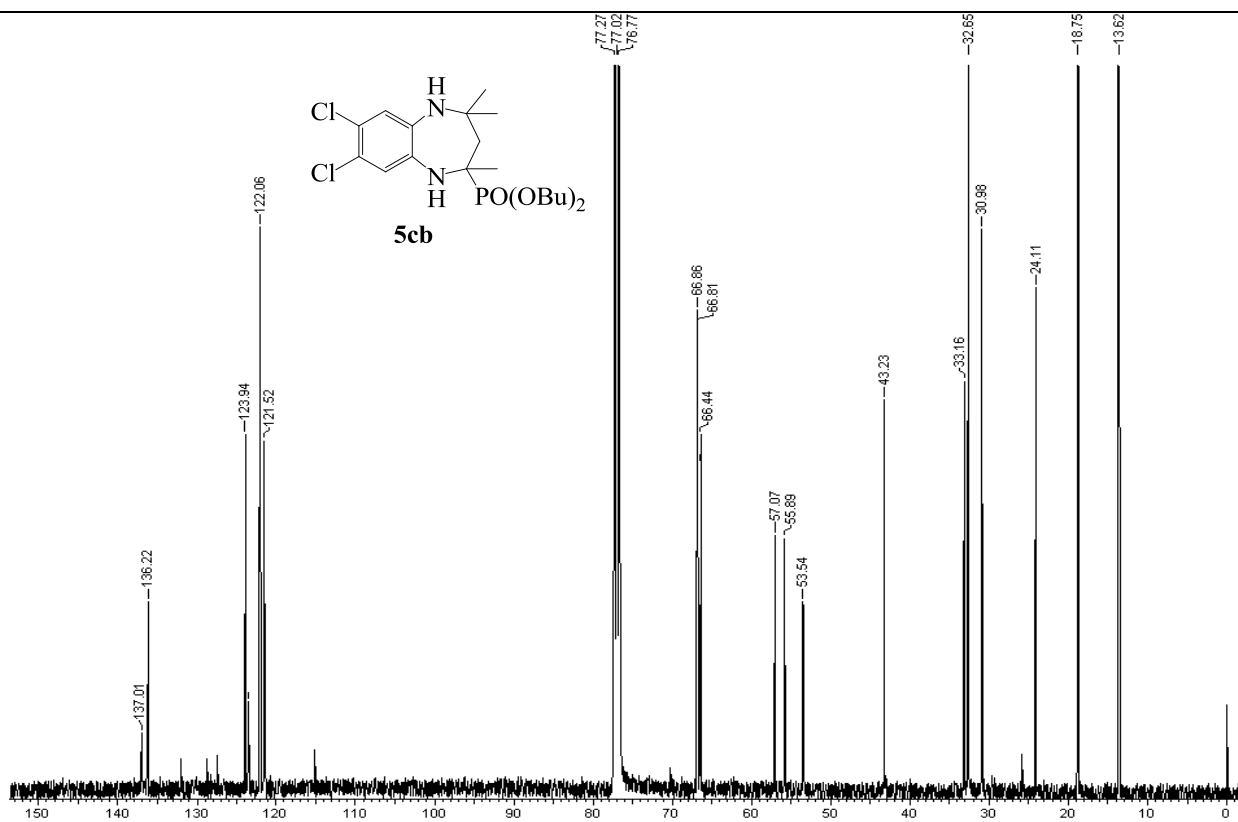
$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) of **5ba**+**5ba'** $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ) of **5ba**+**5ba'**

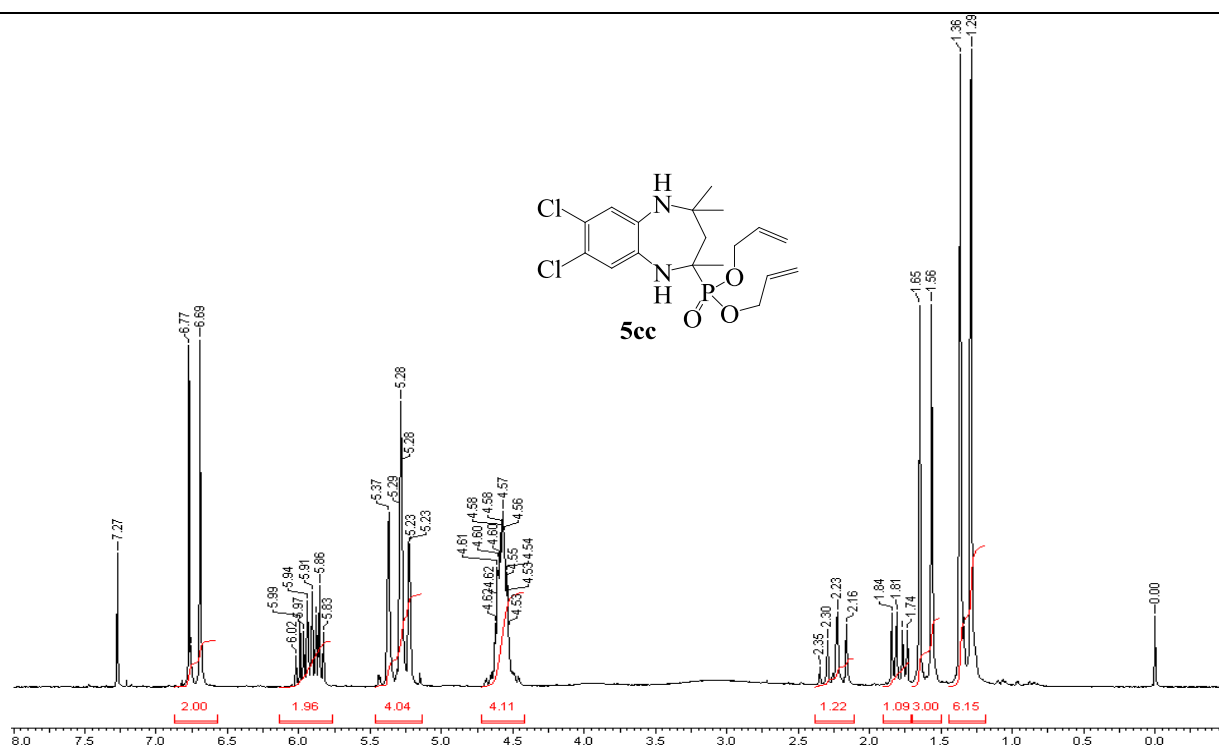
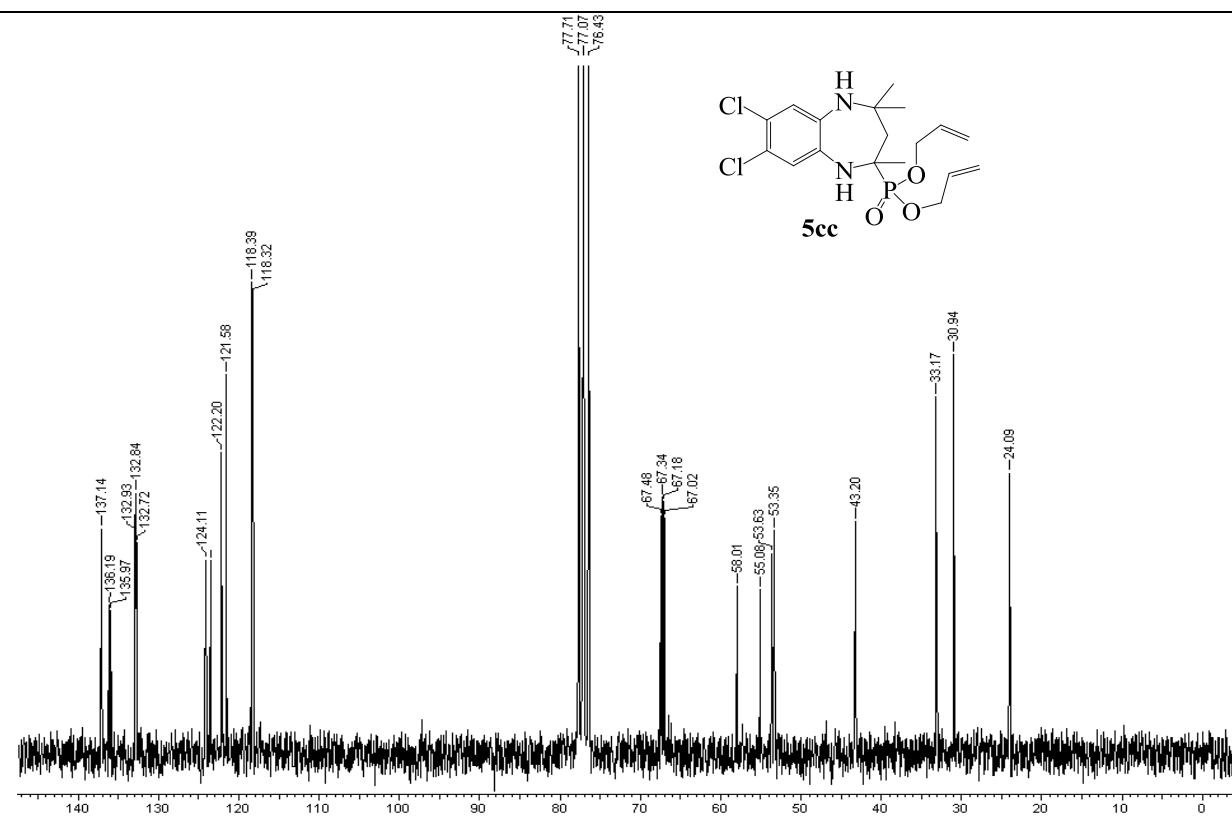
<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) of **5bb**+**5bb'**<sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) of **5bb**+**5bb'**

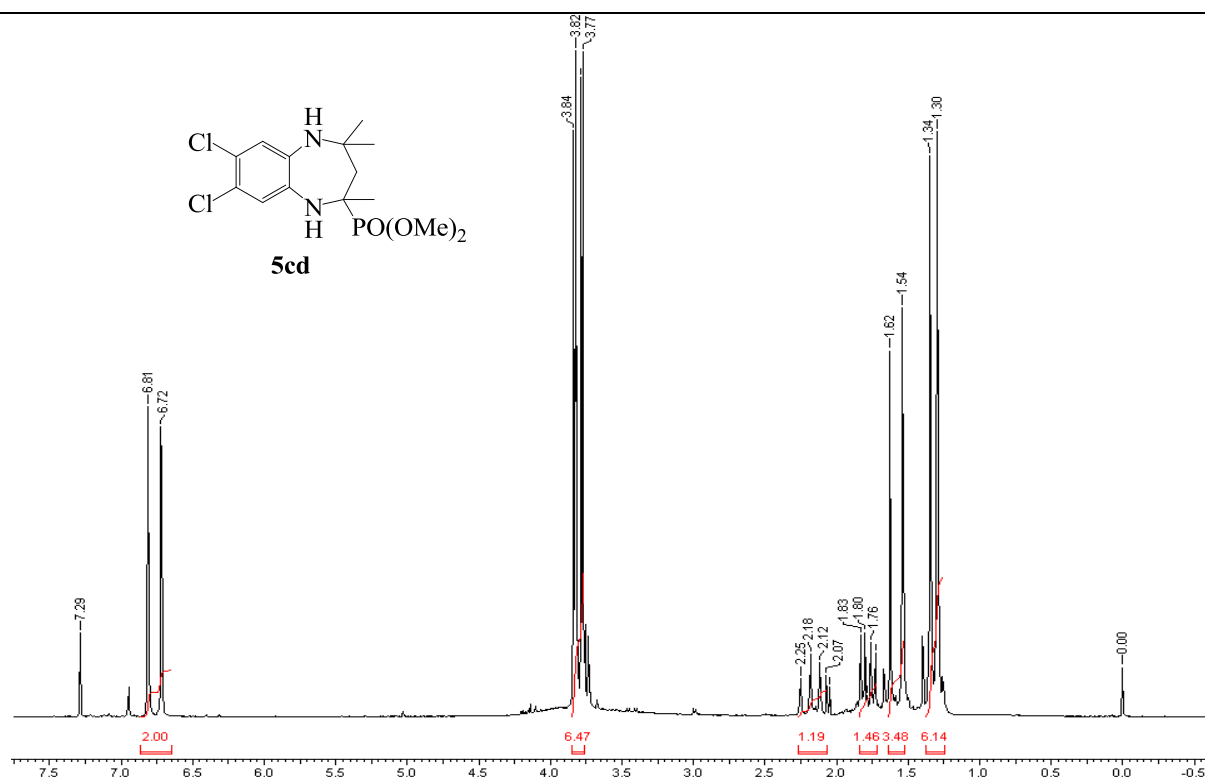
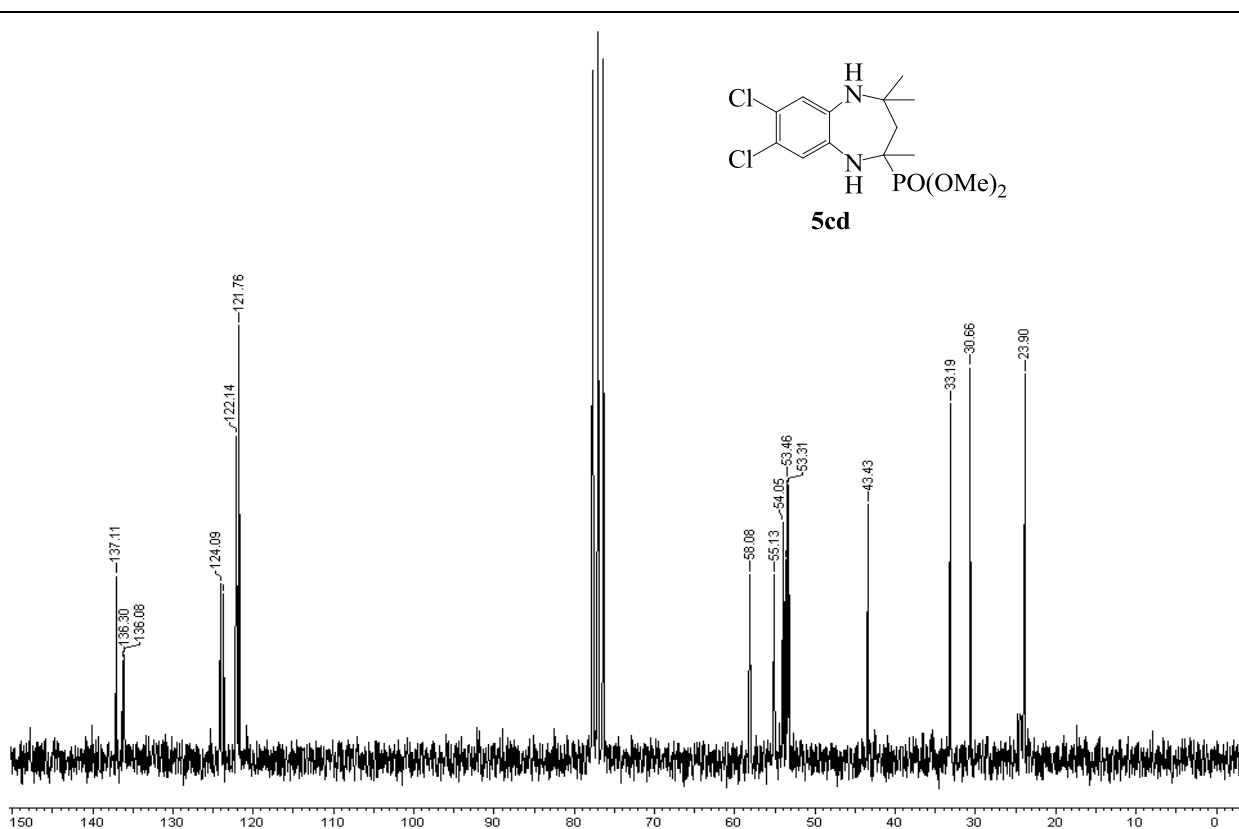
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **5bc**+**5bc'** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **5bc**+**5bc'**

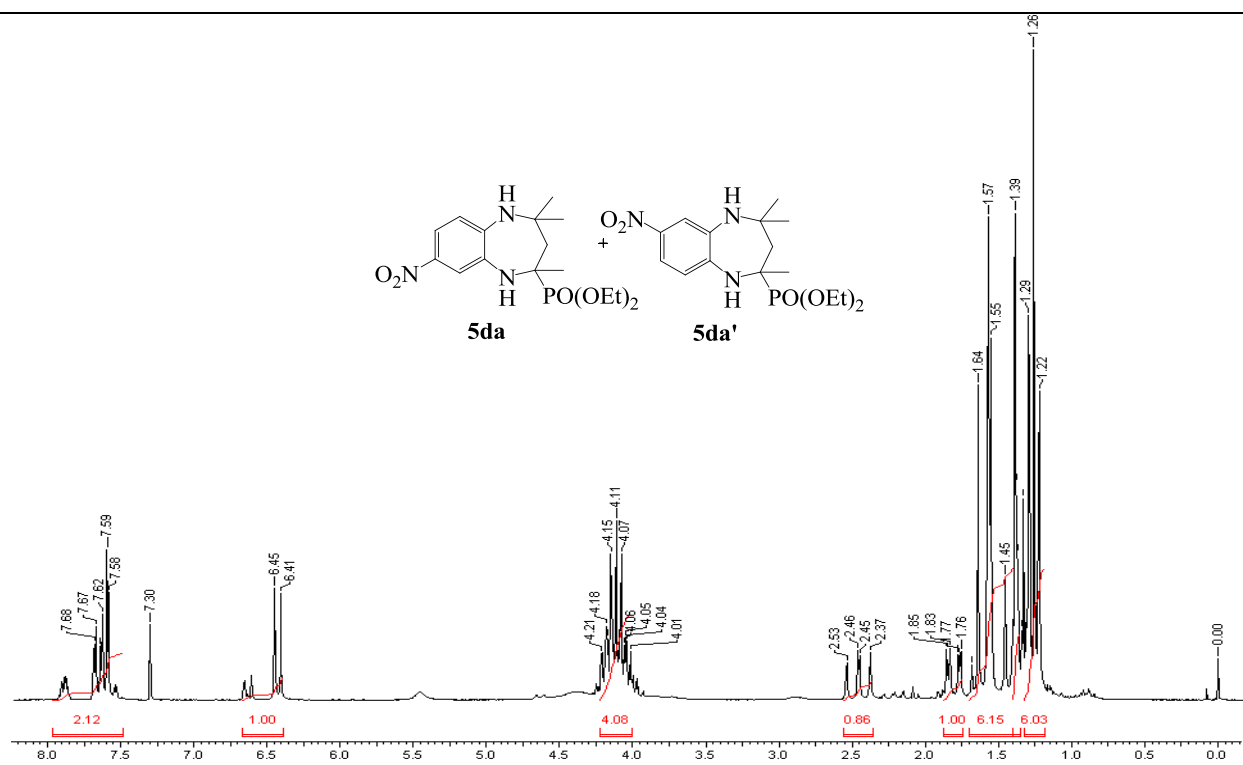
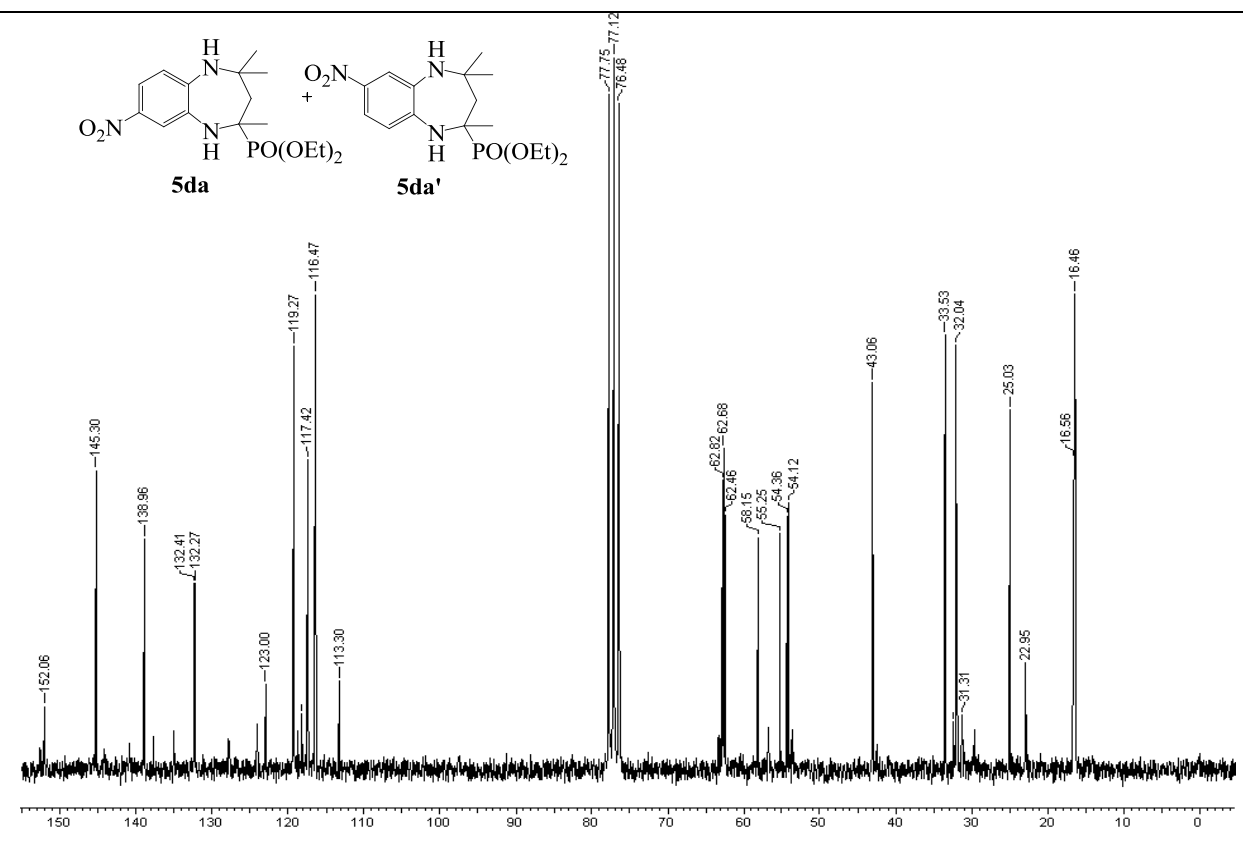


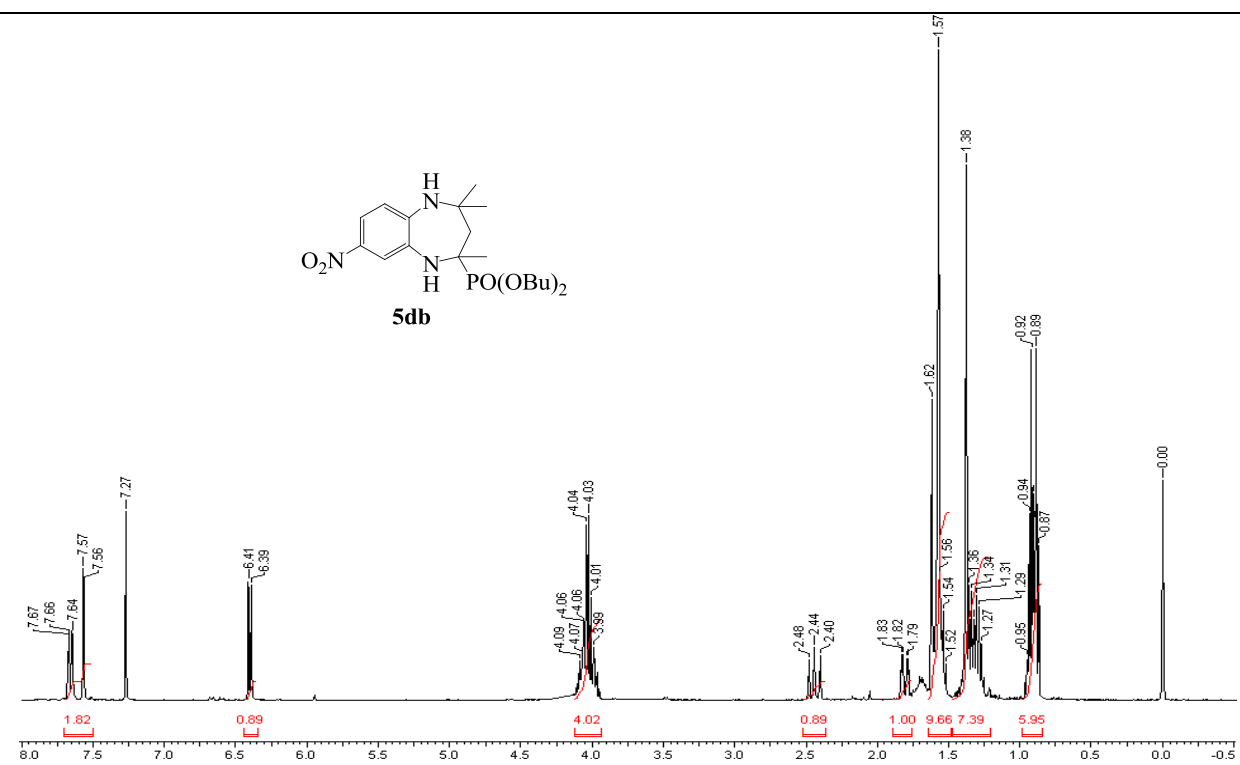
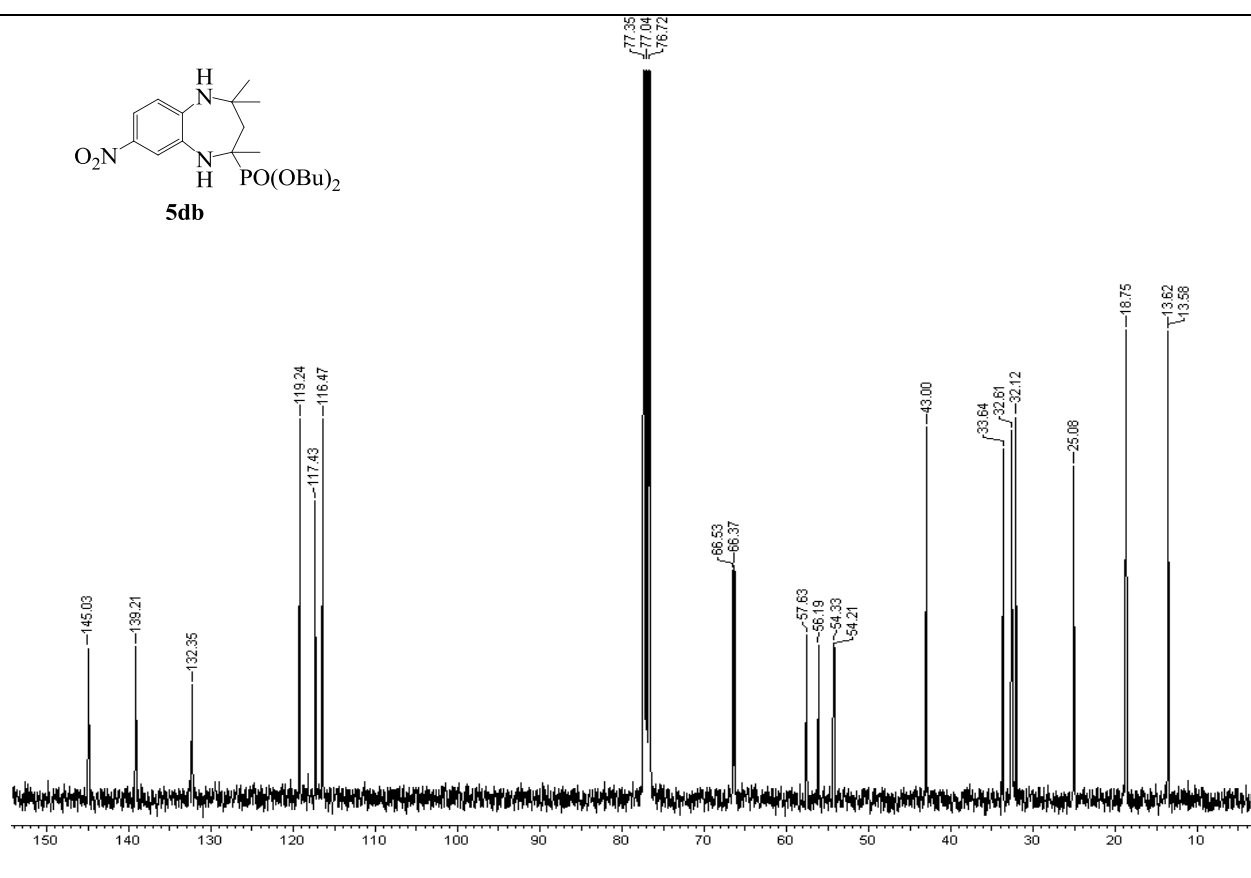
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) of **5ca** $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) of **5ca**

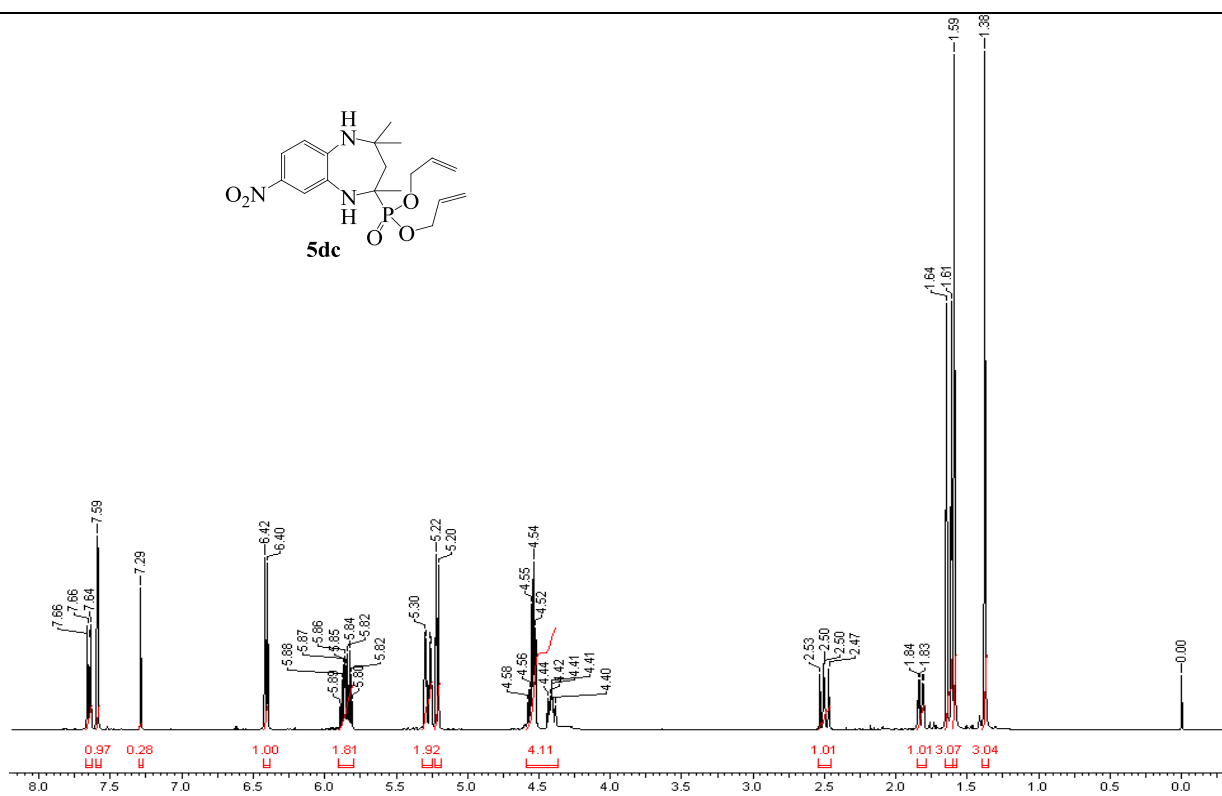
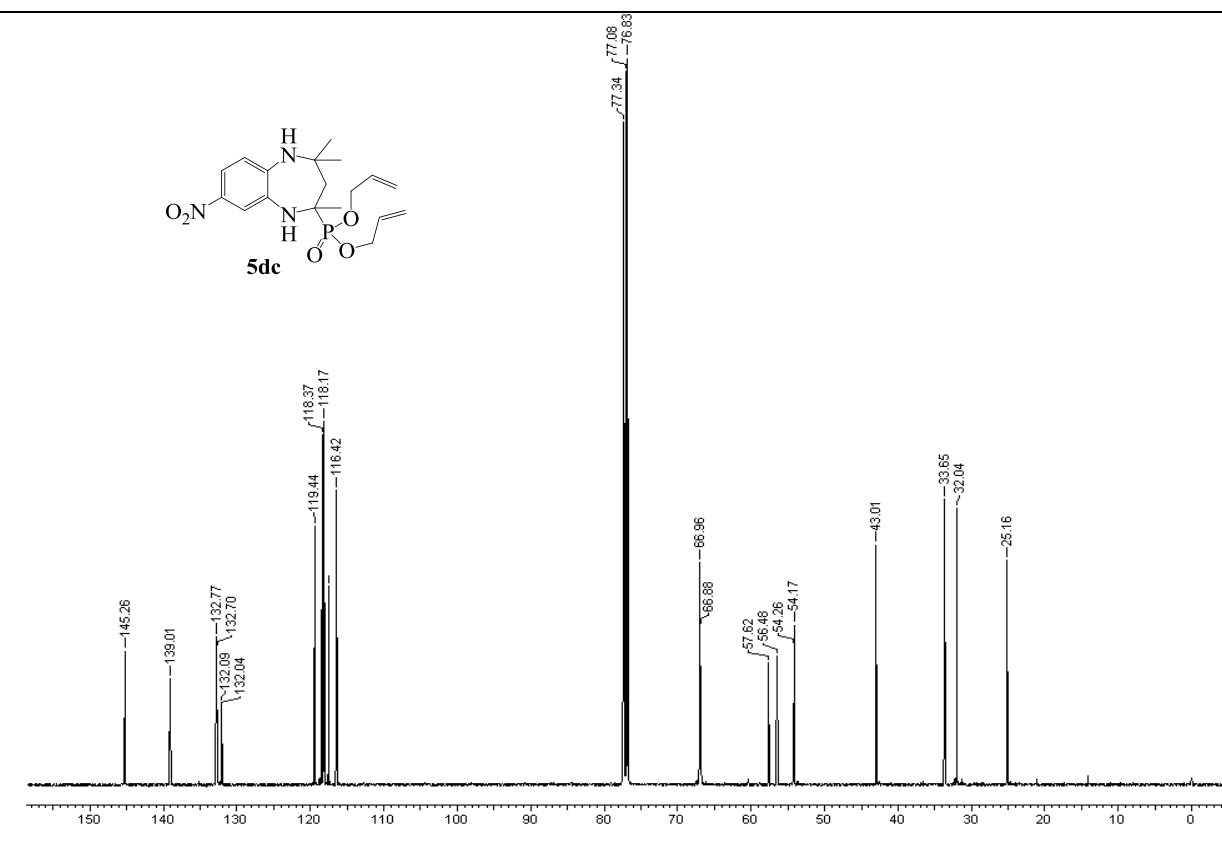
$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) of **5cb**+**5cb'** $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ) of **5cb**+**5cb'**

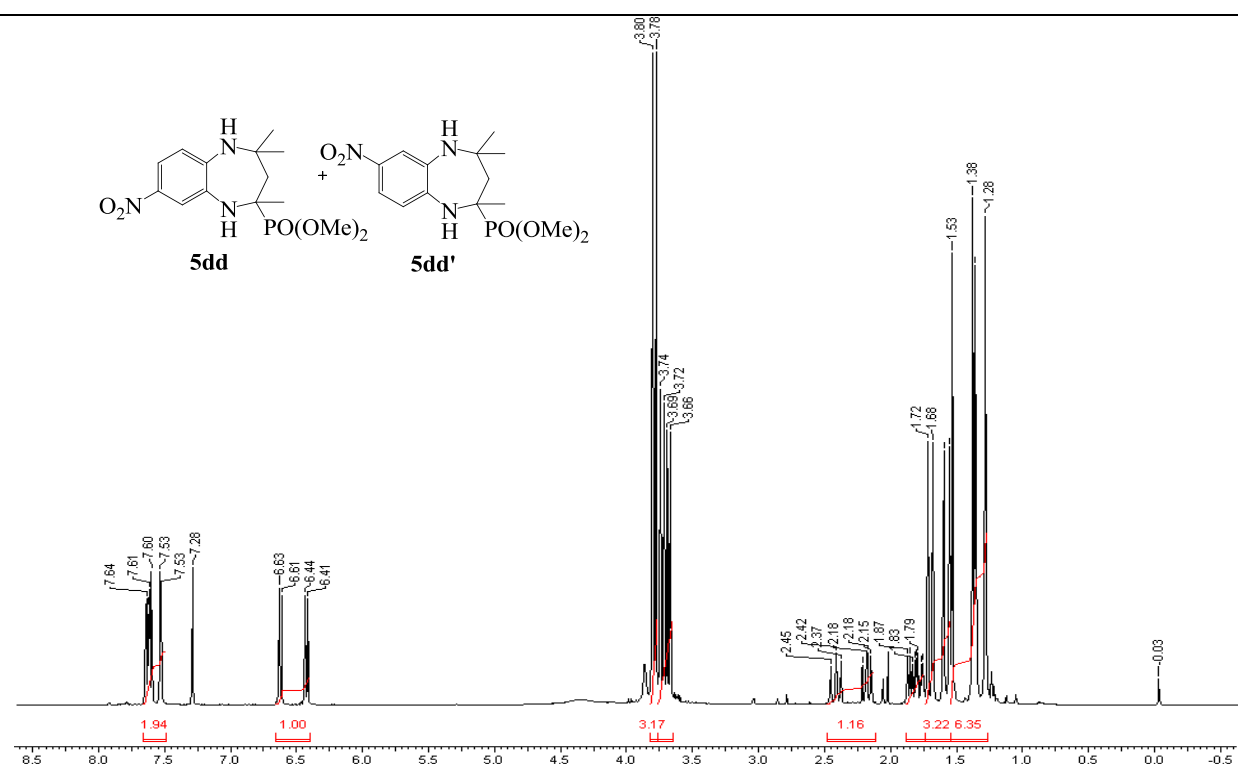
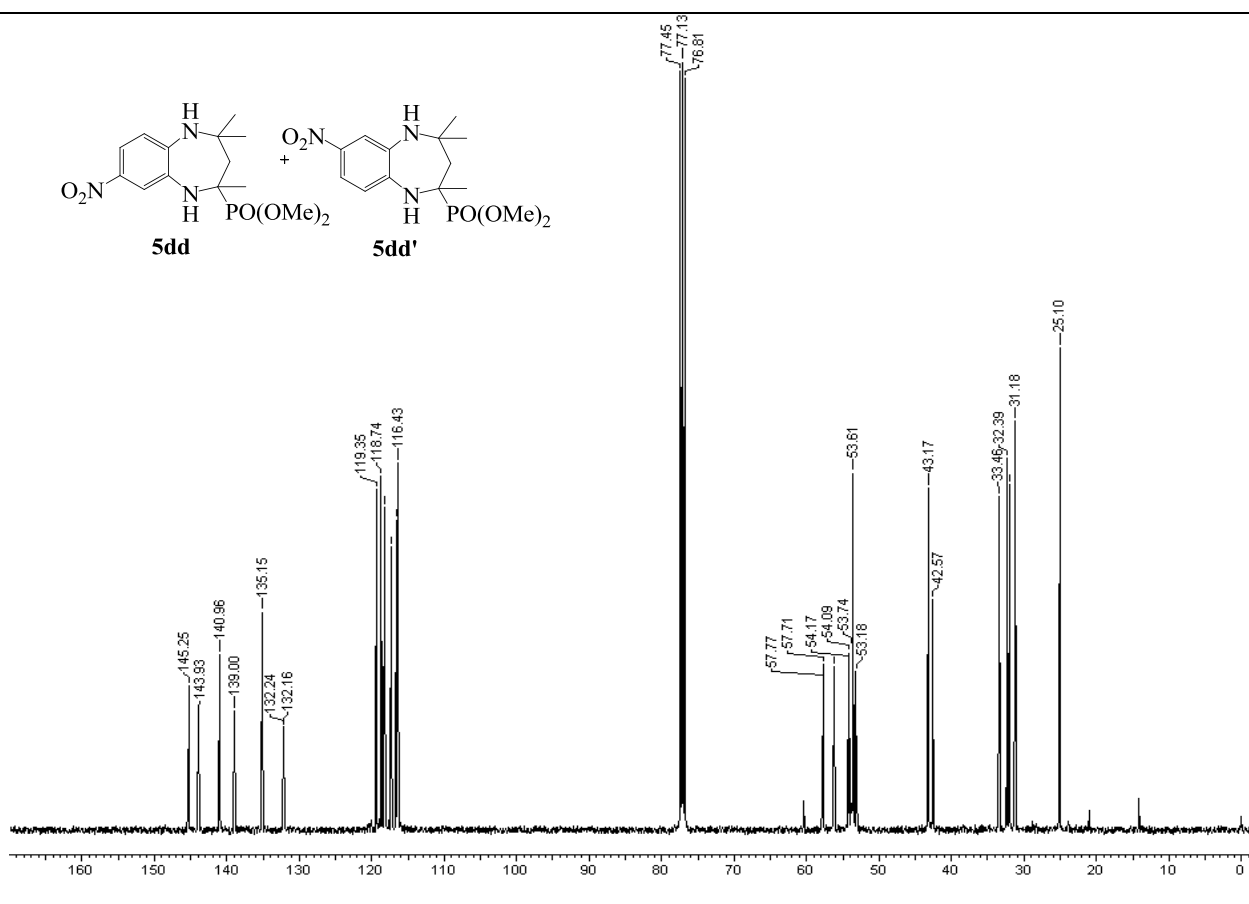
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) of **5cc** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **5cc**

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **5cd** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **5cd**

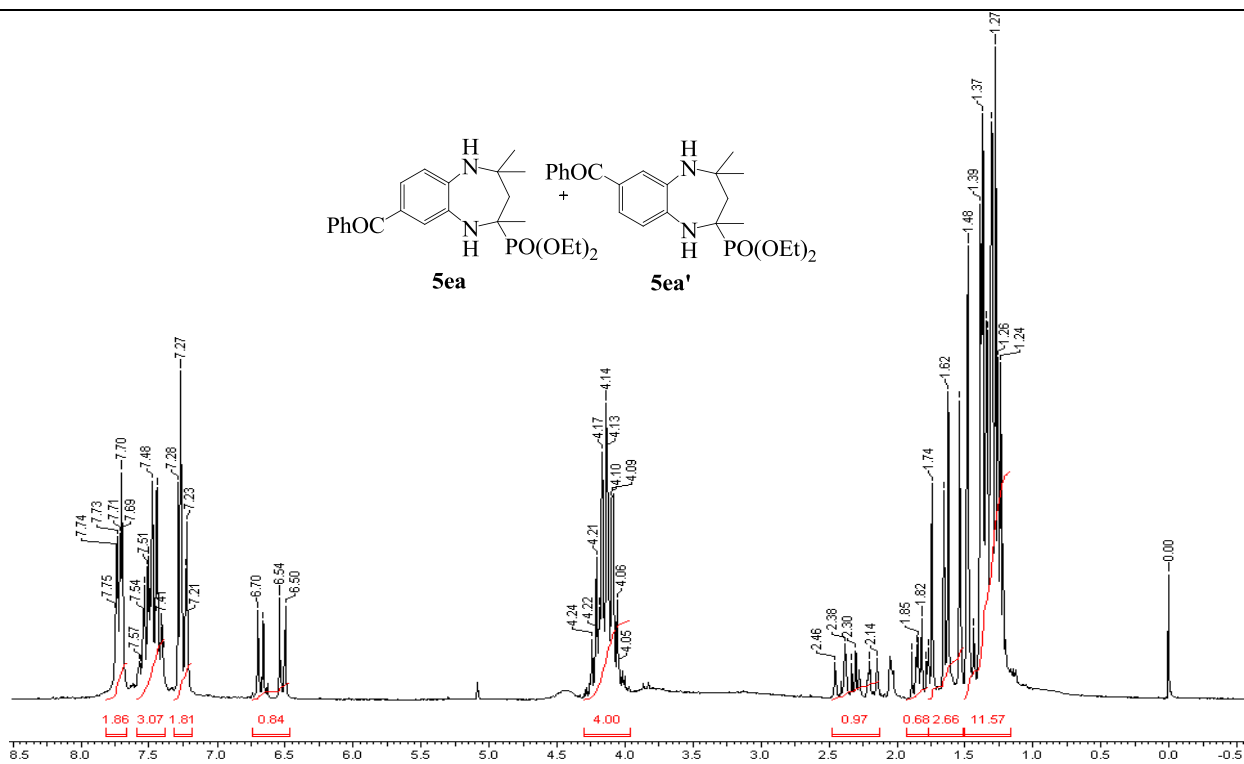
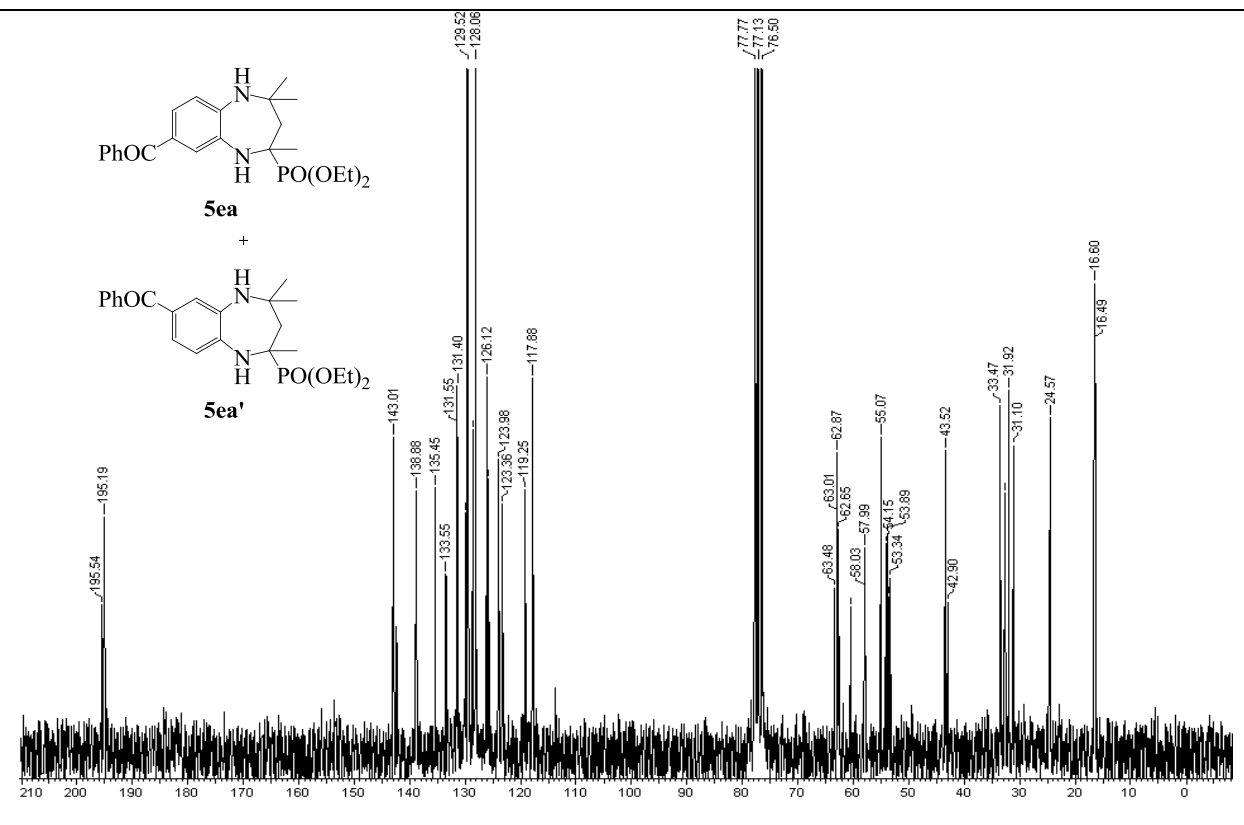
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **5da**+**5da'** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **5da**+**5da'**

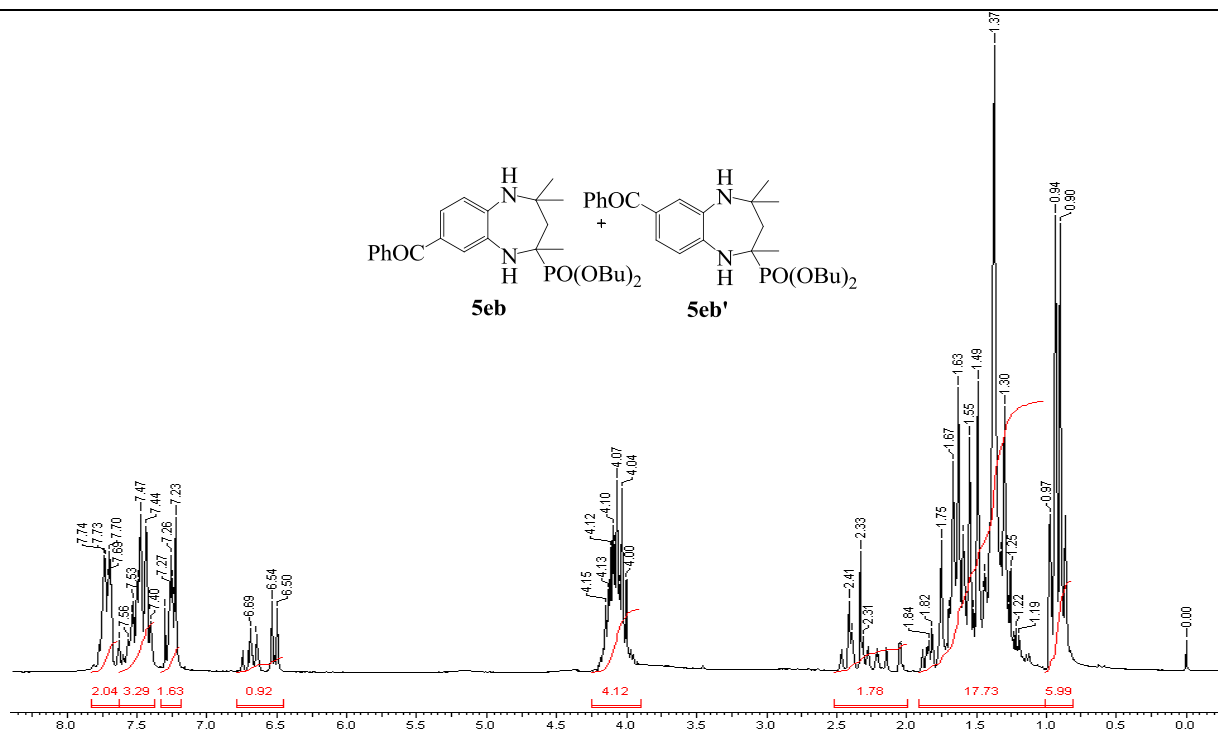
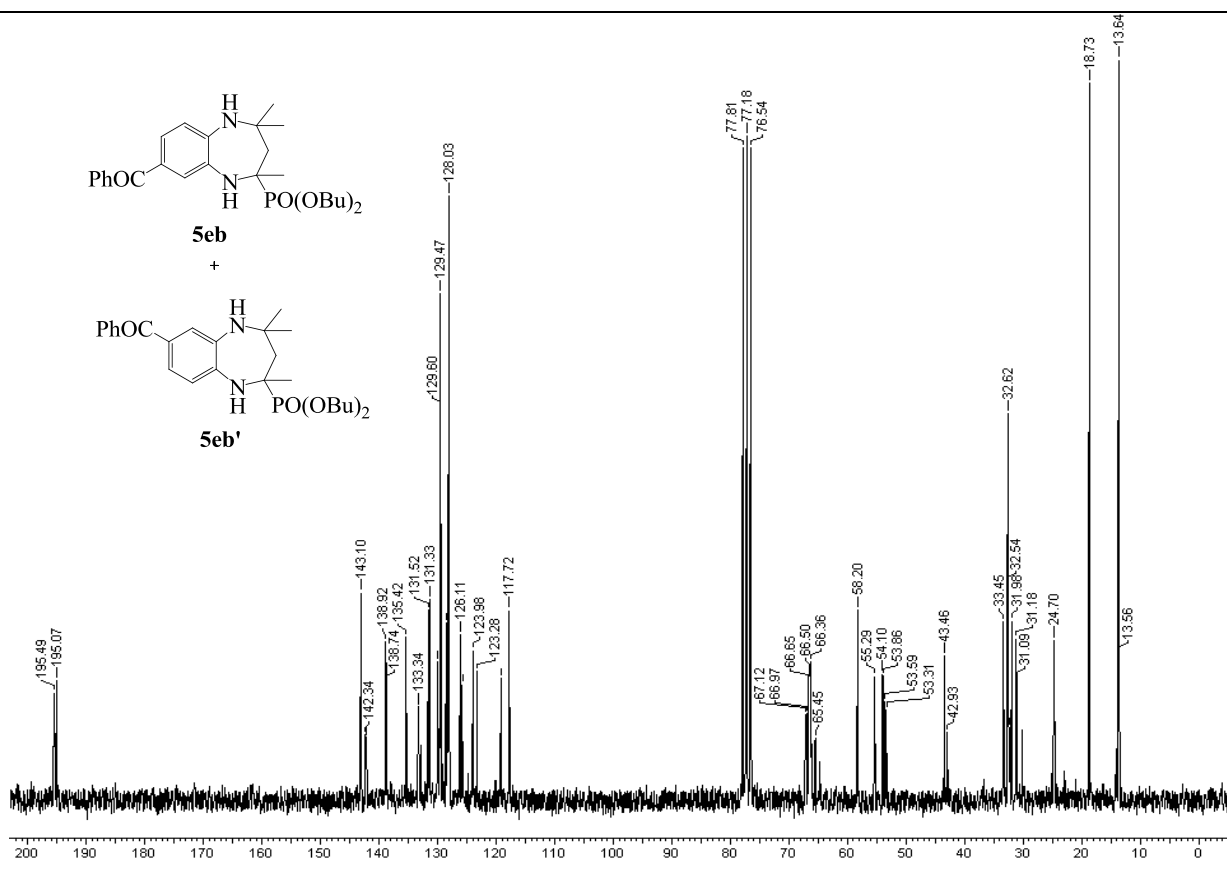
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) of **5db** $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) of **5db**

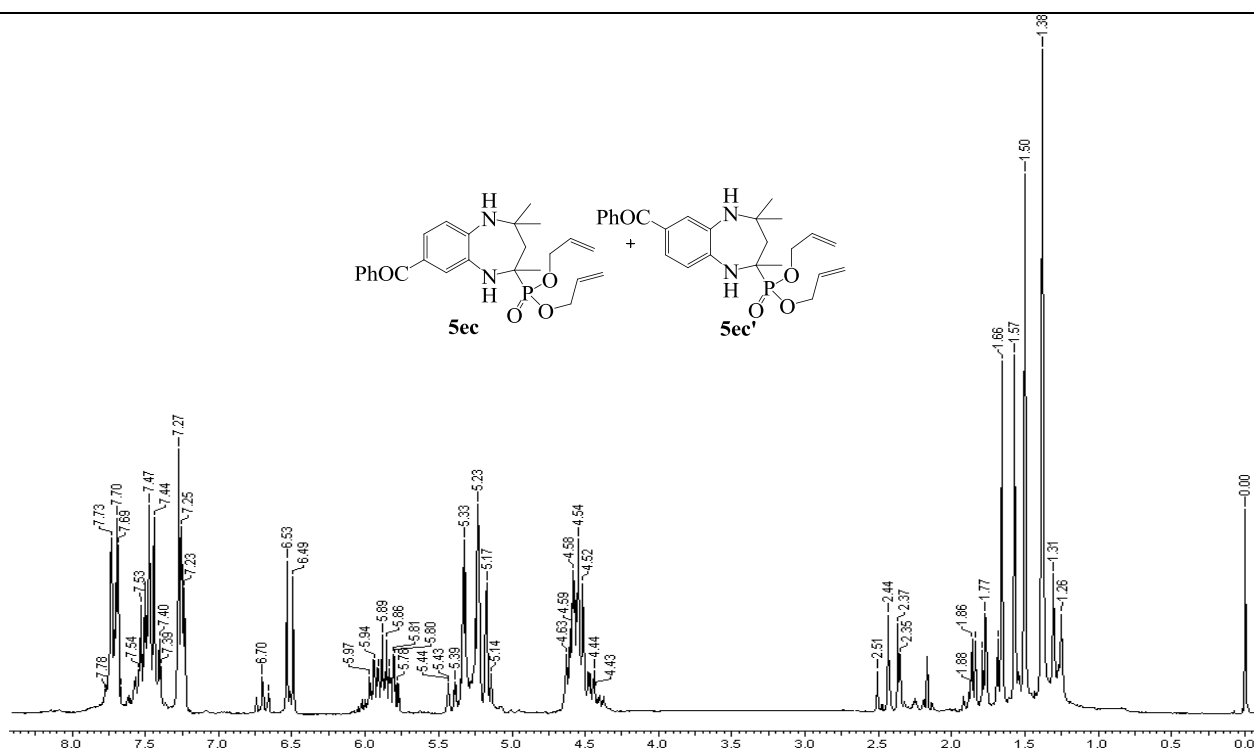
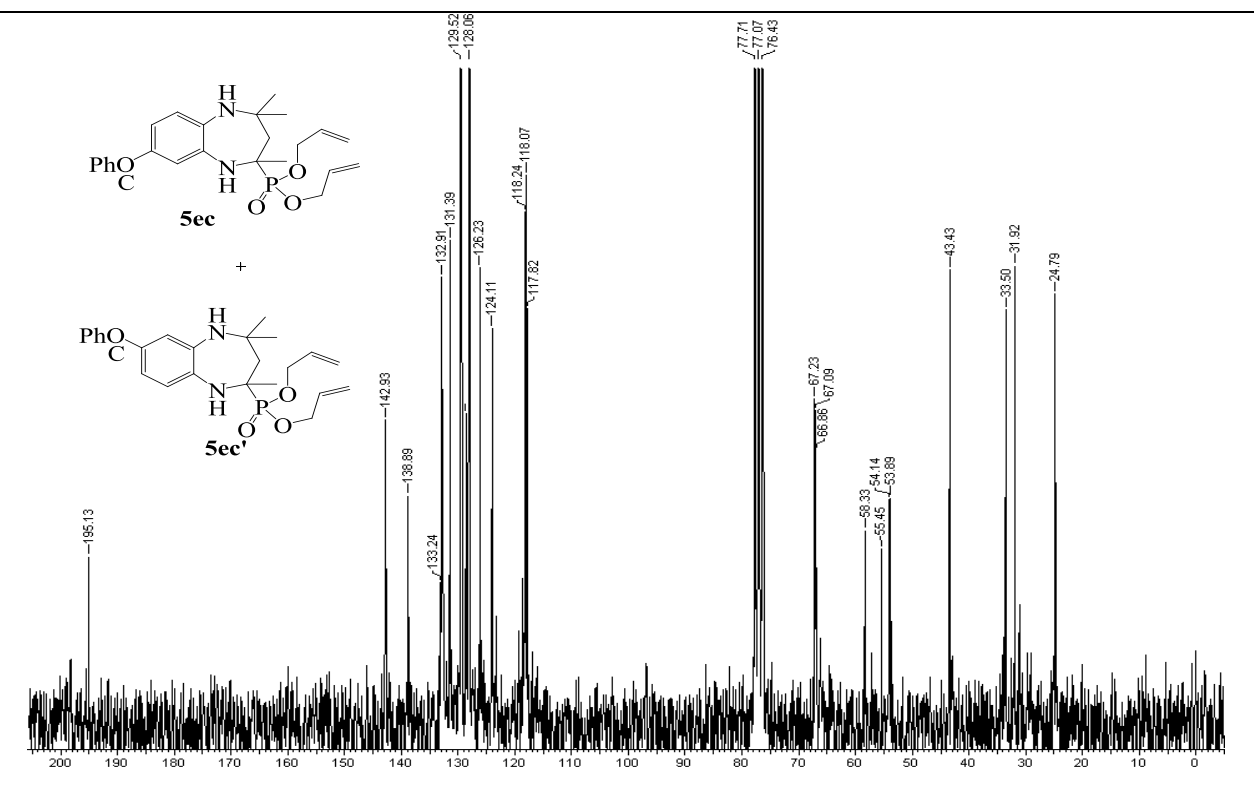
$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) of **5dc** $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ) of **5dc**

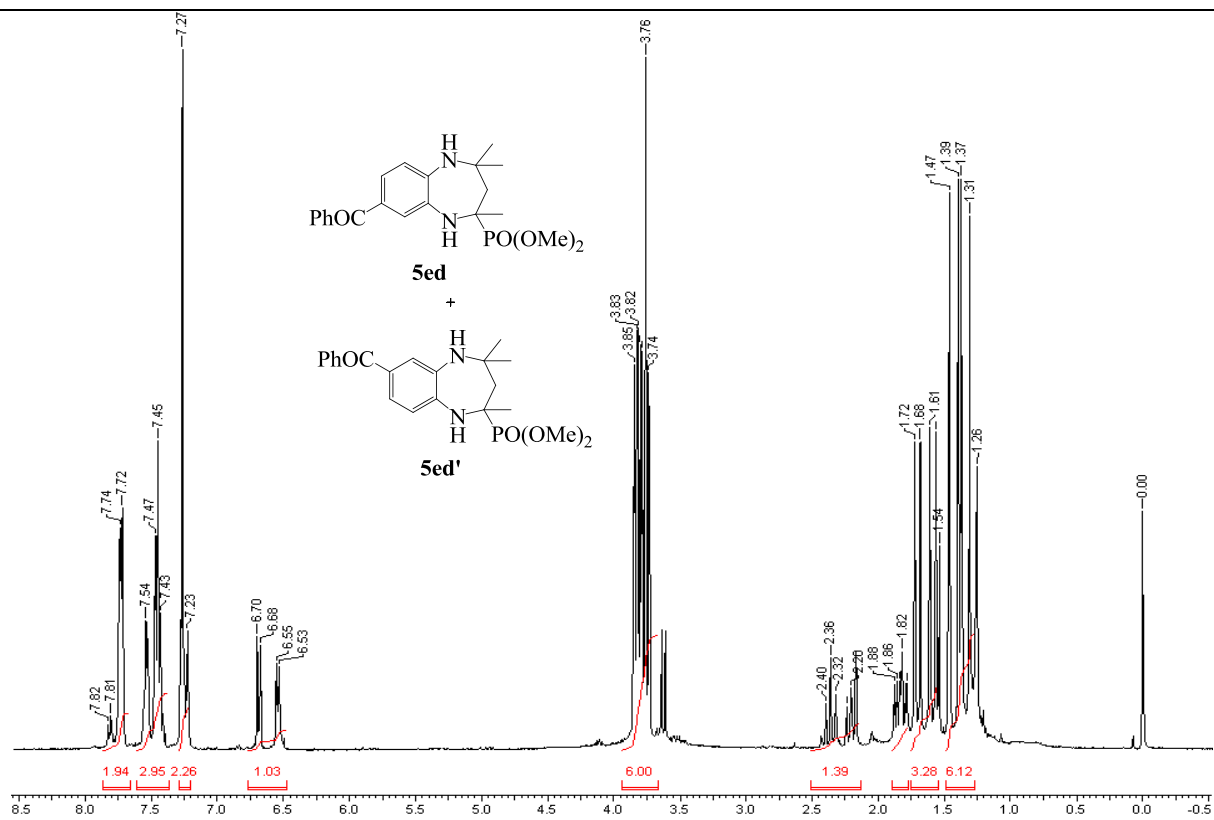
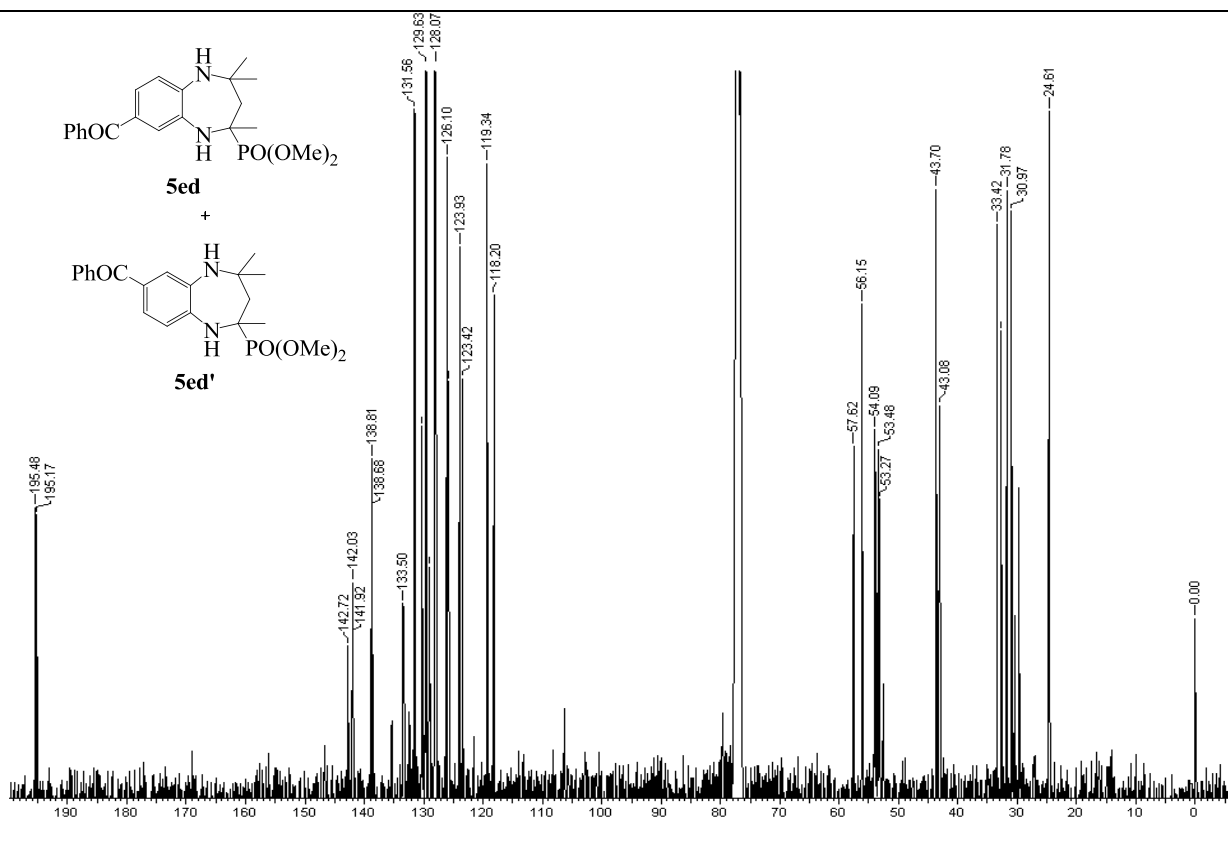
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) of **5dd**+**5dd'** $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) of **5dd**+**5dd'**



$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **5ea**+**5ea'** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **5ea**+**5ea'**

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **5eb**+**5eb'** $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) of **5eb**+**5eb'**

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ) of **5ec**+**5ec'** $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ) of **5ec**+**5ec'**

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) of **5ed+5ed'**<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) of **5ed+5ed'**

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## **Chapter 4**

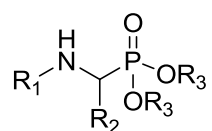
### **Development of New Synthetically Useful Methodologies**

#### **Section B**

**Amberlite-IR 120 Catalyzed Three-Component Synthesis of  $\alpha$ -Aminophosphonates in One-Pot**

## Introduction

$\alpha$ -Aminophosphonates (Figure 1) are considered to be the structural analogues of ester of  $\alpha$ -amino acids and transition-state mimics of peptide hydrolysis, therefore have found immense importance as novel pharmacophore in medicinal chemistry.<sup>1</sup> Phosphonic and carboxylic acid groups differ considerably with respect to shape, size, and acidity.  $\alpha$ -Aminophosphonic acids occupy an important place among the various compounds containing a P–C bond and an amino group, because they are analogues of natural  $\alpha$ -amino acids, the ‘building blocks’ of peptides and proteins. The replacement of acid group by phosphonic acid group has attracted particular interest and has reached a position of eminence in fields of research directed towards discovery, understanding and modification of physiological processes in living organisms.<sup>1</sup>

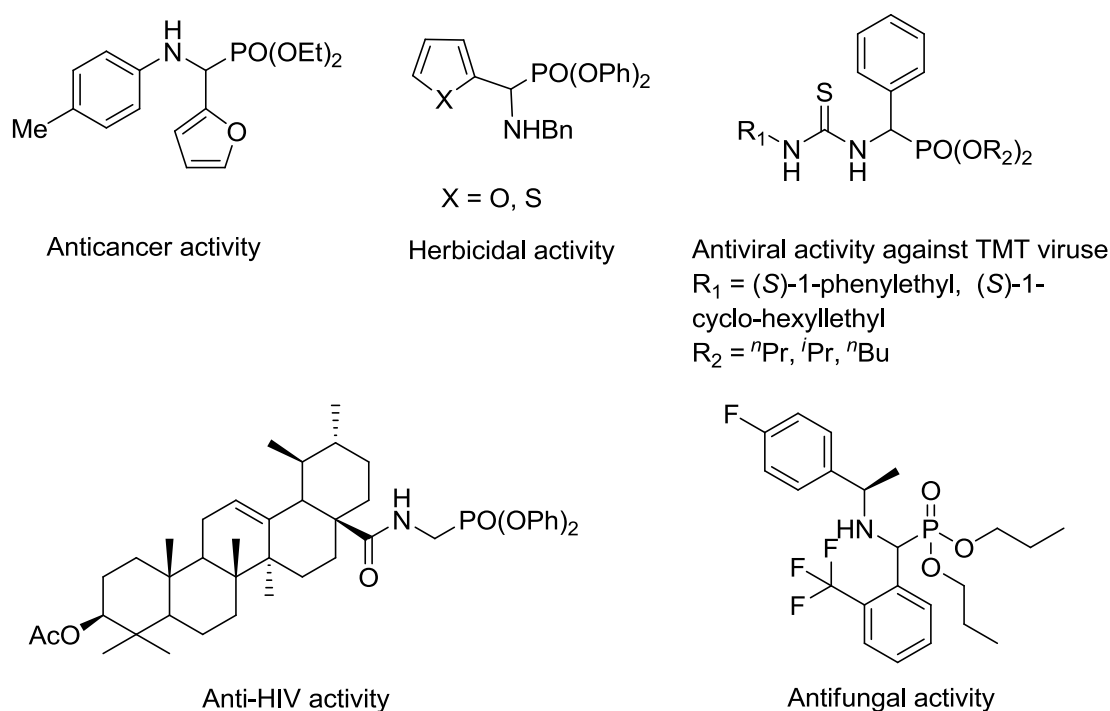


R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> = alkyl or aryl

**Figure 1.** Generalize structure of  $\alpha$ -aminophosphonates.

The replacement<sup>1</sup> of carbon by phosphorus has a number of important consequences such as (a) an additional substituent group is present in the molecule (hydroxyl), (b) the central atom (phosphorus) has a tetrahedral configuration whereas the carbonyl atom is planar and (c) there are significant differences in steric bulk and in acidity ( $pK_a$ ). The tetrahedral configuration of phosphorus has important implications in the design of transition-state analogue enzyme inhibitors which have been exploited in medicinal chemistry. Therefore,  $\alpha$ -aminophosphonates shows various biological activities.<sup>2</sup> In general, low mammalian toxicity of these compounds renders them attractive to be used in agriculture as well as in medicine.<sup>3</sup> These have been reported to be antifungal, pesticidal, herbicidal, haptens of catalytic antibodies, antibiotics and plant growth regulatory activities (Figure 2). These have also been found to act as inhibitors of specific enzymes such as HIV protease, thrombin and human collagenase, and to suppress the growth of various tumours and viruses.<sup>4</sup>

Moreover, some aminophosphonic acids inhibit bone resorption, delay the progression of bone metastases, exert direct cytostatic effects on a variety of human tumour cells and have found clinical applications in the treatment of bone disorders and various cancers.<sup>5</sup> In the past three decades, there has been a growing interest in the chemistry of  $\alpha$ -aminophosphonates with an emphasis on their synthesis and diverse biological activities.<sup>6</sup> Therefore, the synthesis of  $\alpha$ -aminophosphonates has gained tremendous importance in synthetic organic chemistry.



**Figure 2.** Biological importance of  $\alpha$ -aminophosphonates.

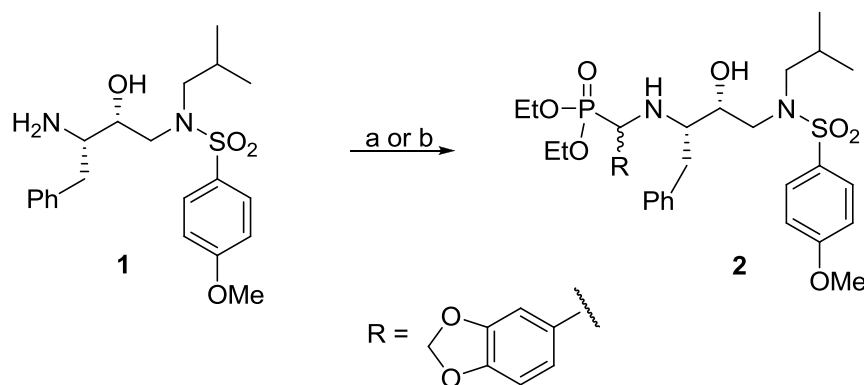
## Present Work

Various synthetic approaches have been reported for the synthesis of  $\alpha$ -aminophosphonates however, nucleophilic addition reaction of phosphites with imines is one of the most preferred methods, which is usually catalyzed by an alkali metal alkoxide *e.g.* NaOEt or Lewis acids<sup>7</sup> such as ZnCl<sub>2</sub>, SnCl<sub>2</sub>, SnCl<sub>4</sub>, BF<sub>3</sub>.Et<sub>2</sub>O and MgBr<sub>2</sub>. In recent times several catalysts have been reported for the one pot synthesis of  $\alpha$ -aminophosphonates which includes tetramethylguanidine,<sup>8a</sup> dodecatungstophosphoric acid,<sup>8b</sup> silica-gel,<sup>8c</sup> magnesium perchlorate,<sup>8d</sup> TiCl<sub>4</sub>,<sup>8e</sup> FeCl<sub>3</sub>,<sup>8f</sup> YbCl<sub>3</sub>,<sup>8g</sup> [bmim]Cl-AlCl<sub>3</sub> ionic liquid,<sup>8h</sup> montmorillonite K10,<sup>8i</sup> bismuth(III)

chloride,<sup>8j</sup> gallium triiodide,<sup>8k</sup> LiClO<sub>4</sub>,<sup>8l</sup> CAN,<sup>8m</sup> samarium diiodide,<sup>8n</sup> CF<sub>3</sub>COOH,<sup>8o</sup> TaCl<sub>5</sub>-SiO<sub>2</sub>,<sup>8p</sup> indium(III) chloride<sup>8q</sup> and zirconium(IV) compounds<sup>8r</sup> etc. However, these reactions can not proceed in one-pot from a carbonyl compound, an amine and a phosphite because the water that is generated during the course of reaction can decompose or deactivate the Lewis acid. This drawback has been well taken care of by one of our recent methods developed in our research group utilizing bismuth nitrate pentahydrate as an efficient Lewis acid catalyst as it is able to tolerate trace amounts of water.<sup>9</sup> However, most of these catalytic systems are applicable for the three component reaction of aromatic amines, aromatic aldehydes and phosphites. The Kabachnik-Field reaction of aliphatic amine, aldehyde and phosphite is difficult. Only InCl<sub>3</sub>,<sup>8q</sup> montmorillonite KSF,<sup>8i</sup> lithium perchlorate,<sup>8l</sup> Mg(ClO<sub>4</sub>)<sub>2</sub>,<sup>8d</sup> and ZrO(ClO<sub>4</sub>)<sub>2</sub>.6H<sub>2</sub>O<sup>8r</sup> listed above are known to effect the Kabachnik-Field reaction with aliphatic amines. Moreover, during the synthesis of aminophosphonate derivatives of hydroxyethylamine isostere (HEA) (see Chapter 2) we carried out Kabachnik-Field reaction of amine **1** with piperonal and diethylphosphite under the influence of various reported catalyst, however it did not give corresponding  $\alpha$ -aminophosphonates. Therefore, we thought to develop the method for the synthesis of  $\alpha$ -aminophosphonates which will be applicable to also aliphatic amines *e.g.* HEA isostere by using environmentally benign and reusable catalyst.

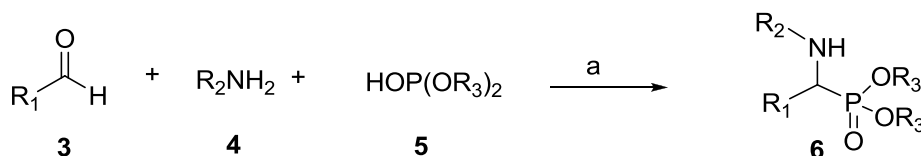
## Results and Discussion

We carried out Kabachnik-Fields reaction of the amine **1** with piperonal and diethylphosphite using known literature methods<sup>8</sup> that include catalytic systems like TaCl<sub>5</sub>, InCl<sub>3</sub>, and Mg(ClO<sub>4</sub>)<sub>2</sub> under various reaction conditions however, none of them gave the corresponding product **2** (Scheme 1). The reaction was limited up to the formation of imine and unreacted starting material (~60%) was recovered even when the reaction was continued after 24 h. When reaction was repeated in the presence of Amberlite-IR 120 (acidic) under neat reaction condition, the traces of product formation was observed after 24 h. We repeated the same reaction under microwave irradiation and the corresponding product **2** was obtained in good yield within 1 minute as a diastereomeric mixture (1:1).



**Scheme 1.** Reagents and conditions: (a)  $\text{TaCl}_5$  or  $\text{InCl}_3$  or  $\text{Mg}(\text{ClO}_4)_2$ ,  $\text{RCHO}$ , diethyl phosphite; (b)  $\text{RCHO}$ , diethyl phosphite, MWI, Amberlite-IR 120 (acidic), 53%.

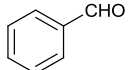
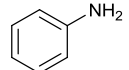
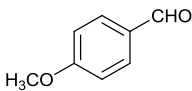
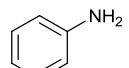
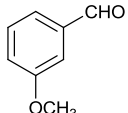
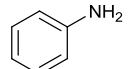
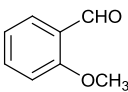
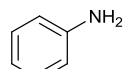
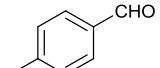
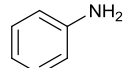
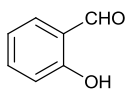
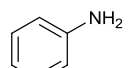
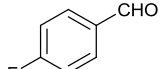
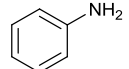
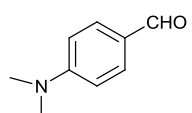
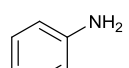
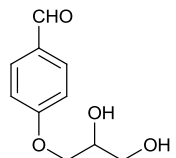
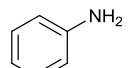
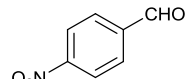
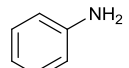
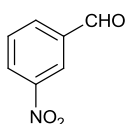
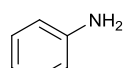
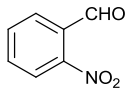
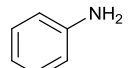
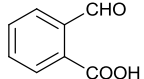
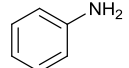
Therefore, we opined that Amberlite-IR 120 under microwave irradiation will be a good alternative for Kabachnik-Fields reaction. Thus, we generalized this method by utilizing diverse carbonyl compounds (aldehydes and ketones), amines (aliphatic and aromatic) and phosphites (Scheme 2). The results are summarized in Table 1.

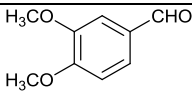
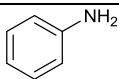
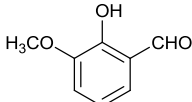
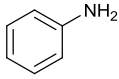
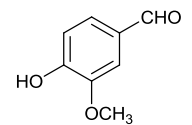
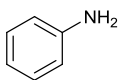
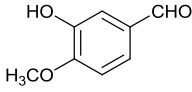
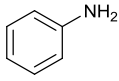
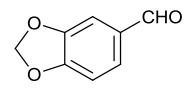
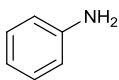
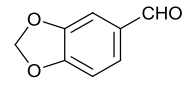
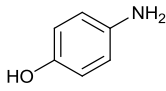
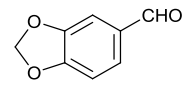
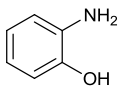
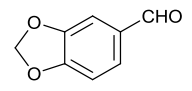
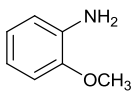
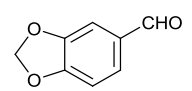
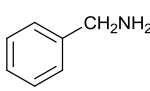
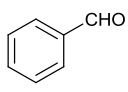
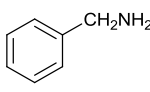
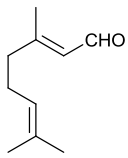
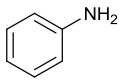
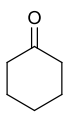
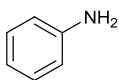
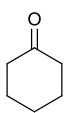
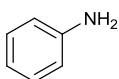


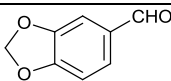
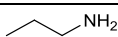
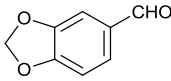

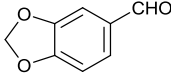
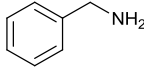
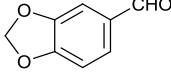

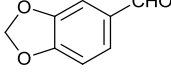
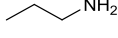
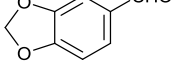
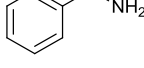
**Scheme 2.** Reagents and conditions: (a) MWI, Amberlite-IR 120 (acidic).

Several structurally diverse carbonyl compounds and aniline/substituted aniline were subjected to this novel procedure and corresponding  $\alpha$ -amino phosphonates were obtained in high to excellent yields. The results are summarized in Table 1. In all cases, the three-component reaction proceeded smoothly to furnish the corresponding  $\alpha$ -amino phosphonates. The presence of electron-donating groups in the aldehyde furnished the corresponding product in low yields and the reaction was sluggish, however, aldehyde possessing electron-withdrawing group afforded corresponding product in shorter reaction time and in higher yields. Also, amine possessing electron-donating group gave corresponding product in low yield and longer reaction time were required for the completion of the reaction. However, in case of conjugated aldehyde (entry 24), the corresponding product was obtained in poor yield. This method is also applicable for the Kabachnik-Field reaction of alipha-

**Table 1.** One-pot synthesis of  $\alpha$ -aminophosphonates catalyzed by Amberlite-IR 120.

Entry	R-CHO	Phosphites	R <sup>1</sup> -NH <sub>2</sub>	Product	Time (min)	Yield (%) <sup>a</sup>
1		DEP		<b>4a</b>	2	90
2		DEP		<b>4b</b>	5	81
3		DEP		<b>4c</b>	1	92
4		DEP		<b>4d</b>	1	87
5		DEP		<b>4e</b>	1	89
6		DEP		<b>4f</b>	1.5	91
7		DEP		<b>4g</b>	1	87
8		DEP		<b>4h</b>	3	70
9		DEP		<b>4i</b>	2	67
10		DEP		<b>4j</b>	1	95
11		DEP		<b>4k</b>	2.5	88
12		DEP		<b>4l</b>	3	75
13		DEP		<b>4m</b>	1	78

Entry	R-CHO	Phosphites	R <sup>1</sup> -NH <sub>2</sub>	Product	Time (min)	Yield (%) <sup>a</sup>
14		DEP		<b>4n</b>	2	90
15		DEP		<b>4o</b>	2	95
16		DEP		<b>4p</b>	2	90
17		DEP		<b>4q</b>	2	87
18		DEP		<b>4r</b>	2	92
19		DEP		<b>4s</b>	3	98
20		DEP		<b>4t</b>	2	79
21		DEP		<b>4u</b>	2	91
22		DEP		<b>4v</b>	1	81
23		DEP		<b>4w</b>	1	87
24		DEP		<b>4x</b>	1	11
25		DEP		<b>4y</b>	3	78
26		DEP		<b>4z</b>	2	84

Entry	R-CHO	Phosphites	R <sup>1</sup> -NH <sub>2</sub>	Product	Time (min)	Yield (%) <sup>a</sup>
27		DEP		<b>4aa</b>	2	41
28		DEP		<b>4ab</b>	2	64
29		DBP		<b>4ac</b>	7	71
30		DBP		<b>4ad</b>	4	55
31		DBP		<b>4ae</b>	4	48
32		DAP		<b>4af</b>	2	58

<sup>a</sup>Yields refer to pure isolated product.

DEP = diethyl phosphite; DBP = dibutyl phosphite; DAP = diallyl phosphite

-atic amines. The wide applicability of the present method is evident from the fact that it is tolerant towards various functional groups present in the substrates *e.g.* alkoxy, halides, nitro, methylenedioxy, carboxylic and hydroxy groups. Moreover, the catalyst could be reused without affecting the yield of the desired product and reaction time thus, making it environmentally benign.

## Conclusion

In conclusion, Amberlite-IR 120 was found to be an efficient catalyst in one-pot reaction of aldehydes, amines, and diethyl phosphite to afford  $\alpha$ -amino phosphonates in good to excellent yields.<sup>10</sup> The main advantages of the present synthetic protocol are: mild, solvent free conditions, ecofriendly catalyst and easy reaction work-up procedure. Further, present method could be useful for substrates bearing wide variety of functional groups. It is expected that the present methodology will find much better application in organic synthesis due to low cost, non-toxic nature and reusability of the catalyst.

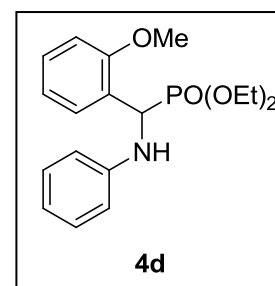


## Experimental

**Typical experimental procedure:** Carbonyl compound (1 mmol), amine (1 mmol), diethylphosphite (1 mmol) and Amberlite IR 120 (100 mg) were taken in a Pyrex test tube and exposed to microwave irradiation (Kenstar Model No. OM-9918C; 2450 MHz, 2350 W) for appropriate time (see Table 1). After completion of the reaction (TLC), the reaction mixture was cooled and DCM (25 mL) was added. The catalyst was filtered out from the reaction mixture and filtrate was concentrated under vacuum. The residue was chromatographed over silica gel column (100-200 mesh) and eluted with ethyl acetate-petroleum ether (3:17 to 3:7) to afford the corresponding pure  $\alpha$ -aminophosphonates.

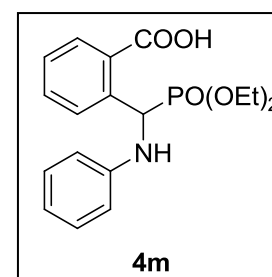
### Diethyl (2-methoxyphenyl)(phenylamino)methylphosphonate (4d).

Solid; mp 98-99 °C;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  1.13 (t,  $J = 7.2$  Hz, 3H), 1.28 (t,  $J = 7.1$  Hz, 3H), 3.77 (s, 3H), 4.19-3.63 (m, 4H), 4.70 (d,  $^1J_{\text{PH}} = 23.0$  Hz, 1H), 7.41-6.56 (m, 9H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  16.3 (d,  $^3J_{\text{PC}} = 5.5$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 16.5 (d,  $^3J_{\text{PC}} = 5.5$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 55.2, 55.3 (d,  $^1J_{\text{PC}} = 152.2$  Hz,  $-\text{CHP}$ ), 63.23 (d,  $^2J_{\text{PC}} = 7.0$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 63.37 (d,  $^2J_{\text{PC}} = 7.3$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 114.0, 114.1, 118.4, 127.7, 128.9, 129.0, 113.9, 129.2, 146.5, 159.3; MS (ESI):  $m/z$  388  $[\text{M}+\text{K}]^+$ , 372  $[\text{M}+\text{Na}]^+$ , 350  $[\text{M}+\text{H}]^+$ ; Anal. Calcd for  $\text{C}_{18}\text{H}_{24}\text{NO}_4\text{P}$ : C, 61.88; H, 6.92; N, 4.01. Found: C, 61.09; H, 6.86; N, 3.95%.



### 2-((Diethoxyphosphoryl)(phenylamino)methyl)benzoic acid (4m).

Solid; mp 97-98 °C;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  0.94 (m, 6H), 3.92-3.44 (m, 4H), 5.57 (d,  $^1J_{\text{PH}} = 12.8$  Hz, 1H), 7.99-7.29 (m, 9H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  15.8 (d,  $^3J_{\text{PC}} = 6.9$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 16.0 (d,  $^3J_{\text{PC}} = 5.1$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 59.2 (d,  $^1J_{\text{PC}} = 153.7$  Hz,  $-\text{CHP}$ ), 62.6 (d,  $^2J_{\text{PC}} = 7.3$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 63.8 (d,  $^2J_{\text{PC}} = 7.0$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 124.4, 124.6,

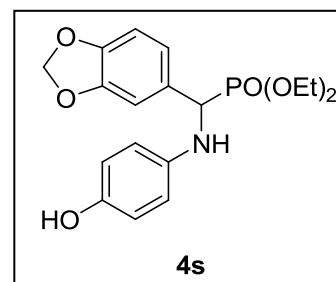


125.2, 126.5, 128.8, 129.2, 132.0, 132.2, 137.4, 138.0, 167.6; MS (ESI):  $m/z$  384  $[M-H_2O+K]^+$ , 368  $[M-H_2O+Na]^+$ , 346  $[M-H_2O+H]^+$ ; Anal. Calcd for  $C_{18}H_{22}NO_5P$ : C, 59.50; H, 6.10; N, 3.85. Found: C, 59.15; H, 6.02; N, 3.78%.

**Diethyl benzo[d][1,3]dioxol-5-yl(4-hydroxyphenylamino)methylphosphonate (4s).**

Solid; mp 168-169 °C;  $^1H$  NMR (200 MHz,  $CDCl_3$ , TMS):

$\delta$  0.98 (t,  $J = 7.1$  Hz, 3H), 1.11 (t,  $J = 7.1$  Hz, 3H), 4.05-3.56 (m, 4H), 4.63 (d,  $^1J_{PH} = 24.3$  Hz, 1H), 5.81 (s, 2H), 6.95-6.42 (m, 7H);  $^{13}C$  NMR (50 MHz,  $CDCl_3$ , TMS):  $\delta$  15.7 (d,  $^3J_{PC} = 5.9$  Hz,  $-OCH_2CH_3$ ); 15.9 (d,  $^3J_{PC} = 5.9$  Hz,  $-OCH_2CH_3$ ), 55.9 (d,  $^1J_{PC} = 153.3$  Hz,  $-CHP$ ), 62.3 (d,  $^2J_{PC}$

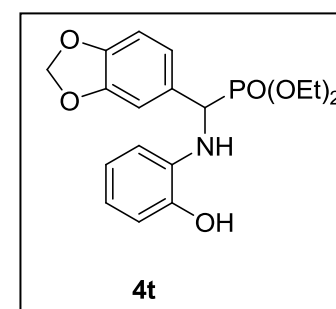


= 7.3 Hz,  $-OCH_2CH_3$ ), 62.5 (d,  $^2J_{PC} = 7.3$  Hz,  $-OCH_2CH_3$ ), 101.1, 107.6, 108.5, 115.4, 115.5, 121.9, 131.1, 140.0, 147.1, 147.7, 149.7; MS (ESI):  $m/z$  418  $[M+K]^+$ , 402  $[M+Na]^+$ , 380  $[M+H]^+$ ; Anal. Calcd for  $C_{18}H_{22}NO_6P$ : C, 56.99; H, 5.85; N, 3.69. Found: C, 56.60; H, 5.73; N, 3.62%.

**Diethyl benzo[d][1,3]dioxol-5-yl(2-hydroxyphenylamino)methylphosphonate (4t).**

Solid; mp 132-133 °C;  $^1H$  NMR (200 MHz,  $CDCl_3$ , TMS):

$\delta$  1.18 (t,  $J = 7.1$  Hz, 3H); 1.29 (t,  $J = 7.1$  Hz, 3H), 4.32-3.69 (m, 4H), 4.79 (d,  $^1J_{PH} = 25.0$  Hz, 1H), 5.87 (s, 2H), 6.97-6.47 (m, 7H);  $^{13}C$  NMR (50 MHz,  $CDCl_3$ , TMS):  $\delta$  16.2 (d,  $^3J_{PC} = 5.9$  Hz,  $-OCH_2CH_3$ ), 16.4 (d,  $^3J_{PC} = 5.9$  Hz,  $-OCH_2CH_3$ ), 55.7 (d,  $^1J_{PC} = 155.9$  Hz,  $-CHP$ ), 63.7 (d,

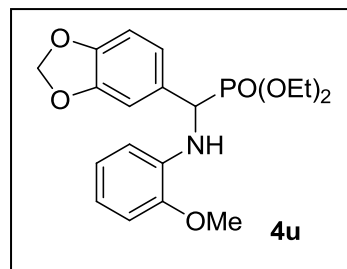


$^2J_{PC} = 7.0$  Hz,  $-OCH_2CH_3$ ), 64.2 (d,  $^2J_{PC} = 7.0$  Hz,  $-OCH_2CH_3$ ), 101.1, 108.3, 108.5, 112.0, 114.3, 118.4, 119.9, 121.6, 129.6, 135.0, 145.3, 147.3, 147.9; MS (ESI):  $m/z$  402  $[M+Na]^+$ , 380  $[M+H]^+$ ; Anal. Calcd for  $C_{18}H_{22}NO_6P$ : C, 56.99; H, 5.85; N, 3.69. Found: C, 56.65; H, 5.77; N, 3.60%.

**Diethyl benzo[d][1,3]dioxol-5-yl(2-methoxyphenylamino)methylphosphonate (4u).**

Syrupy liquid;  $^1H$  NMR (200 MHz,  $CDCl_3$ , TMS):  $\delta$  1.20 (t,  $J = 7.1$  Hz, 3H), 1.28 (t,  $J = 7.2$  Hz, 3H), 3.88 (s, 3H), 4.16-3.85 (m, 4H), 4.67 (dd,  $^1J_{PH} = 24.0$ , 8 Hz, 1H),

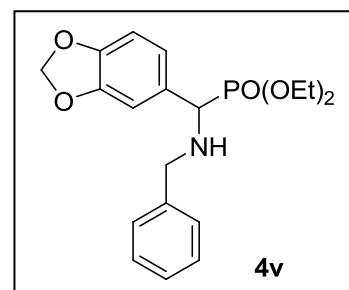
5.27 (t,  $J = 8.0$  Hz, 1H), 5.93 (s, 2H), 6.96-6.39 (m, 7H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  16.3 (d,  $^3J_{\text{PC}} = 5.8$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 16.5 (d,  $^3J_{\text{PC}} = 5.8$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 55.6, 55.6 (d,  $^1J_{\text{PC}} = 152.6$  Hz,  $-\text{CHP}$ ), 63.2 (d,  $^2J_{\text{PC}} = 7.0$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 63.4 (d,  $^2J_{\text{PC}} = 7.0$



Hz,  $-\text{OCH}_2\text{CH}_3$ ), 101.1, 108.2, 108.3, 109.6, 111.2, 117.7, 121.0, 121.4, 129.8, 136.0, 147.3, 147.9, 148.0; MS (ESI):  $m/z$  432  $[\text{M}+\text{K}]^+$ , 416  $[\text{M}+\text{Na}]^+$ , 394  $[\text{M}+\text{H}]^+$ ; Anal. Calcd for  $\text{C}_{19}\text{H}_{24}\text{NO}_6\text{P}$ : C, 58.01; H, 6.15; N, 3.56. Found: C, 57.78; H, 6.10; N, 3.46%.

#### Diethyl benzo[d][1,3]dioxol-5-yl(benzylamino)methylphosphonate (4v).

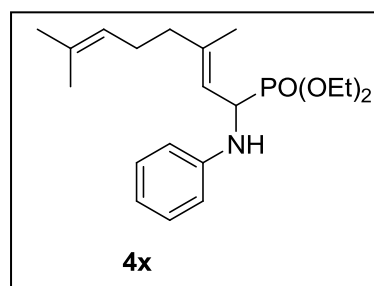
Syrupy liquid;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  1.18 (t,  $J = 7.1$  Hz, 3H), 1.29 (t,  $J = 7.1$  Hz, 3H), 3.52 (d,  $^1J_{\text{PH}} = 13.3$  Hz, 1H), 4.13-3.77 (m, 6H), 5.97 (s, 2H), 7.35-6.77 (m, 8H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  16.4 (t,  $^3J_{\text{PC}} = 6.2, 5.6$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 51.0 (d,  $^3J_{\text{PC}} = 17.6$  Hz,  $-\text{NHCH}_2\text{Ph}$ ), 59.2 (d,  $^1J_{\text{PC}} = 155.2$  Hz,  $-\text{CHP}$ ), 62.9



(t,  $^2J_{\text{PC}} = 6.6, 7.3$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 101.1, 108.2, 108.7, 122.4, 127.2, 128.3, 128.4, 129.4, 139.3, 147.4, 147.9; MS (ESI):  $m/z$  416  $[\text{M}+\text{K}]^+$ , 400  $[\text{M}+\text{Na}]^+$ , 378  $[\text{M}+\text{H}]^+$ ; Anal. Calcd for  $\text{C}_{19}\text{H}_{24}\text{NO}_5\text{P}$ : C, 60.47; H, 6.41; N, 3.71. Found: C, 60.29; H, 6.28; N, 3.62%.

#### (E)-Diethyl 3,7-dimethyl-1-(phenylamino)octa-2,6-dienylphosphonate (4x).

Syrupy liquid;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  1.27-1.17 (m, 6H), 1.49 (s, 3H), 1.50 (s, 3H), 1.61 (s, 3H), 1.72-1.71 (m, 2H), 2.06-1.97 (m, 2H), 4.15-3.97 (m, 4H), 4.38 (dd,  $^1J_{\text{PH}} = 20.7$  Hz,  $^3J_{\text{CH}} = 9.4$  Hz), 5.12-4.95 (m, 2H), 7.13-6.54 (m, 5H);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ , TMS):  $\delta$  16.4, 16.5, 17.1, 17.7, 25.6, 26.2, 39.6,



50.6 (d,  $^1J_{\text{PC}} = 158.5$  Hz,  $-\text{CHP}$ ), 62.8 (d,  $^2J_{\text{PC}} = 7.3$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 63.1 (d,  $^2J_{\text{PC}} = 6.6$  Hz,  $-\text{OCH}_2\text{CH}_3$ ), 113.9, 118.4, 119.9, 123.6, 129.1, 131.8, 141.6, 146.8; MS (ESI):

$m/z$  402  $[M+K]^+$ , 388  $[M+Na]^+$ , 360  $[M+H]^+$ ; Anal. Calcd for  $C_{20}H_{32}NO_3P$ : C, 65.73; H, 8.83; N, 3.83. Found: C, 65.62; H, 8.76; N, 3.72%.

## References

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1. Tebbe's Reagent.  
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  2. Microwave assisted synthesis of 14-aryl-14H dibenzo[a,j]xanthenes catalysed by methanesulphonic acid under solvent-free condition.  
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  3. Amberlite-IR 120 catalyzed three-component synthesis of  $\alpha$ -amino phosphonates in one-pot.  
A. K. Bhattacharya and **K. C. Rana**. *Tetrahedron Lett.* **2008**, 49, 2598.
  4. Synthesis and in vitro study of 14-aryl-14H-dibenzo[a,j]xanthenes as cytotoxic agents.  
A. K. Bhattacharya, **K. C. Rana**, M. Mujahid, I. Sehar and A. K. Saxena. *Bioorg. Med. Chem. Lett.* **2009**, 19, 5590.
  5. An efficient synthesis of benzodiazepinylphosphonates as clostripain inhibitors via FeCl<sub>3</sub>-catalyzed four-component reaction.  
A. K. Bhattacharya, **K. C. Rana**, D. S. Raut, V. P. Mhaindarkar and M. I. Khan (Communicated).
  6. Design and synthesis of aminophosphonate derivatives of hydroxyethylamine (HEA) isostere and their anti-HIV activity.  
A. K. Bhattacharya and **K. C. Rana** (manuscript under preparation).
  7. Design and synthesis of vinylaminophosphonates as cysteine protease inhibitors.  
A. K. Bhattacharya, **K. C. Rana** and M. I. Khan (manuscript under preparation).
  8. Design and synthesis of artemisinin-peptidyl-vinylaminophosphonate hybrid molecules as falcipain-2 protease inhibitors.  
A. K. Bhattacharya, **K. C. Rana** and A. Mohmmmed (manuscript under preparation).
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## *Erratum*

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