SYNTHESIS OF NEW PHOSPHONAMIDES FOR TRANSITION STATE ANALOGUE STUDIES

by

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Abstract

A series of phosphonamidates has been synthesized as substrates for an abzyme approach to the hydrolysis of the side chain of cephalosporin C.

All of the compounds were produced by the coupling of a protected phosphonochloridate with a protected primary amine. The most advanced intermediate synthesized was a protected phosphonamidate of cephalosporin C.

The phosphorous analogues of penicillin G, cephalosporin G and desacetoxycephalosporin G were also synthesized.

Deprotection of benzyl phosphonamidates yielded phosphonamides which were found to be unstable below pH 5. The kinetics of the hydrolysis of one of these phosphonamides were explored at pH 3 and an activation energy of 88 kJ/mol was found.

Dedication

To my parents

and my partner

Acknowledgments

I acknowledge the tremendous intellectual and financial support of Professor Saul Wolfe in helping me to grow in and feel great enthusiasm for the field of organic chemistry. I also acknowledge both Marcy and Dr. Alan Tracy for their excellent assistance in obtaining and interpreting NMR spectra. I thank both Greg Owen and M. K. Yang for providing the mass spectra and micro analyses, respectively.

Furthermore, I thank all of my laboratory colleagues for their help and friendship over the past three years. And finally, I wish to express my gratitude to those members of the Department of Chemistry who have inspired me since my first day at SFU nearly ten years ago.

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List of Abbreviations

THF.....tetrahydrofuran

TMSCl.....chlorotrimethylsilane

DME.....dimethoxyethane

DMF.....dimethylformamide

p-TsCl.....para-toluenesulfonyl chloride

p-TsOH•H_2O.....para-toluenesulfonic acid monohydrate

Ph.....phenyl

Z.....benzyloxycarbonyl

6-APA.....6-aminopenicillanic acid

7-ACA.....7-aminocephalosporanic acid

7-ADCA.....7-aminodesacetoxycephalosporanic acid

AP5.....2-amino-5-phosphonopentanoic acid

TLC.....thin-layer chromatography

HPLC.....high pressure liquid chromatography

COSY.....correlation spectroscopy

Chapter 1

Introduction

1.1 Nomenclature of organic phosphorous compounds

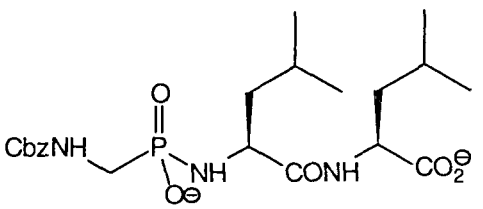
The standard nomenclature for the organophosphorous compounds discussed in this thesis is illustrated with the following examples.

General descriptor	**Specific example**
$P(OR)_3$ trialkyl phosphite	$(PhCH_2O)_2P{-}OSiMe_3$ dibenzyl trimethylsilylphosphite
$(RO)_2P(=O)R$ dialkyl phosphonate	$(PhCH_2O)_2P(=O)CH_2Ph$ dibenzyl benzylphosphonate
$RP(=O)(OH)_2$ phosphonic acid	$PhCH_2P(=O)(OH)_2$ benzylphosphonic acid
$R{-}P(=O)(OR){-}NHR$ alkyl phosphonamidate	$R{-}P(=O)(OCH_2Ph){-}NHR'$ benzyl phosphonamidate
$R{-}P(=O)(OH){-}NHR$ phosphonamide	$R{-}P(=O)(ONa){-}NHR'$ sodium phosphonamide

1.2 Biological applications of phosphonamides

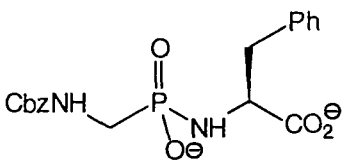
Phosphonamides, the phosphonic acid analogues of amides, have attracted considerable attention in recent years.[1] They act as enzyme inhibitors,[2] peptide mimics[3] and also serve as haptens for the generation of catalytic antibodies (abzymes) for peptide rearrangement[4] (Figure 1.1). A seminal result relating to peptide hydrolysis[5] stimulated the work described here.

P. A. Bartlett, C. K. Marlowe, *Biochem.*, **22** 4618 (1983)



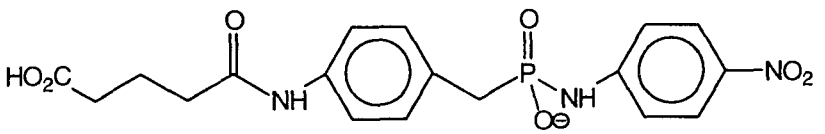
Potent Thermolysin inhibitor

N. E. Jacobsen, P. A. Bartlett, *J.Am.Chem.Soc.*, **103** 654 (1981)



Potent Carboxypeptidase A inhibitor

K. D. Janda, D. Schloeder, S. J. Benkovic, R. A. Lerner, *Science*, **241** 1188 (1988)



Hapten used to elicit a catalytic antibody

Figure 1.1. Examples of the applications of phosphonamides

1.3 Development of antibody catalysis

In 1969 Jencks[6] suggested that antibodies might function as molecular catalysts. This idea has been verified experimentally and developed during the past ten years by the demonstration that monoclonal antibodies designed to bind to hypothetical transition states can catalyze chemical reactions that proceed via these transition states. There are now many publications describing the scope and potential of this concept.[7]

In the past, ambiguities concerning the nature of the species responsible for the catalytic properties of antibodies were attributed to the heterogeneity of the system. These ambiguities have been overcome by use of techniques pioneered by Kohler and Milstein,[8] which allow the large scale preparation of pure monoclonal antibodies.

By mimicking a transition state, a hapten can be regarded as a molecular hand searching out an exactly fitting antibody glove having analogous steric requirements and charge complementarity[9] (Figure 1.2). Once isolated, some of these antibodies may catalyze the specific reaction whose transition state has been simulated. Successful examples include, among others, ester and amide hydrolysis,[10] β-elimination,[11] Diels-Alder cycloaddition,[12] lactonization[13] and photo-induced cleavage.[14]

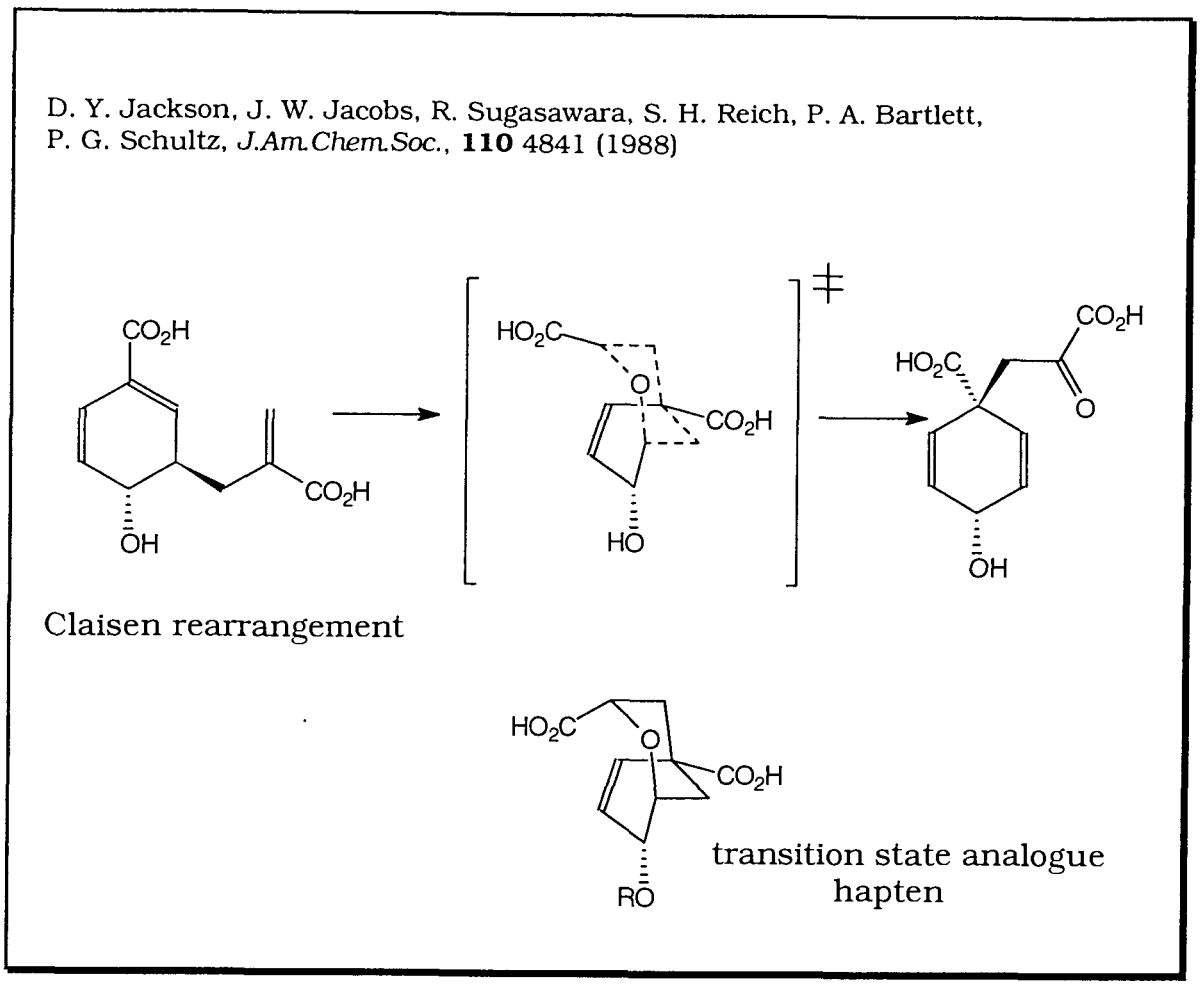


Figure 1.2. The rational design of a transition-state mimic

An elegant consequence of this procedure is that, for any catalytic antibody isolated, the hapten molecule (a stable transition state analogue) will function as a powerful inhibitor of catalysis because of the extraordinary affinity of the antibody for the hapten. The hapten will bind very tightly to the catalytic antibody and block the antibodies' potential to catalyze a reaction on the substrate.

1.4 Synthesis of 7-ACA

Most clinically important β-lactam antibiotics contain the penicillin nucleus **1** or the cephalosporin nucleus **2**, and are prepared from 6-aminopenicillanic acid (6-APA) **3**, 7-aminocephalosporanic acid (7-ACA) **4**, or 7-aminodesacetoxycephalosporanic acid (7-ADCA) **5** (Figure 1.3).

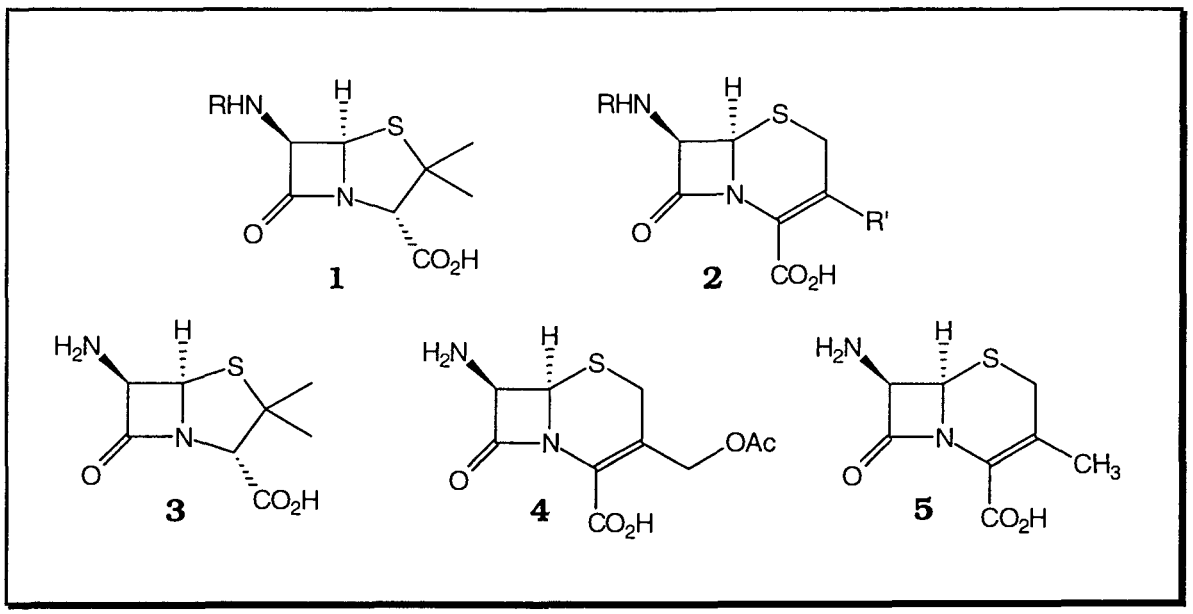


Figure 1.3. The core of penicillin and cephalosporin antibiotics

The biosynthetic pathways leading to penicillins and cephalosporins are well established.[15] In each case, the primary antibiotic is isopenicillin N, **6**, which contains a δ-(L-α-aminoadipyl) side chain (Figure 1.4). In *Penicillium chrysogenum*, there is a deacylation pathway, leading to 6-APA, and also a transacylation pathway, leading to penicillin G, or penicillin V. Because of their hydrophobic properties below pH 4, penicillin G and/or penicillin V are easily isolated from fermentations,

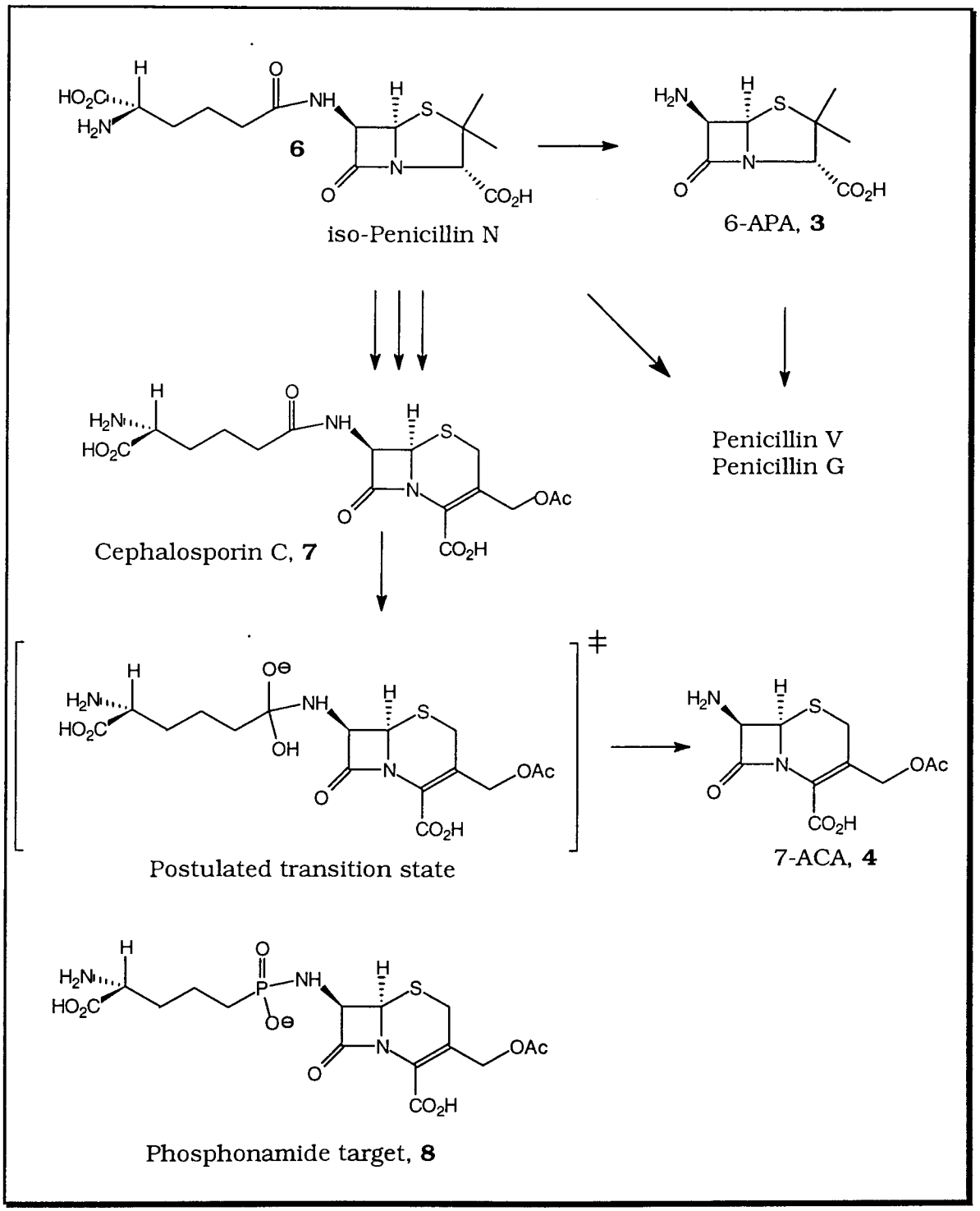


Figure 1.4. Outline of the synthesis of 7-ACA

and approximately 15,000 tons per annum of these penicillins are produced industrially at a cost of $30/kg.[16] One or the other of these antibiotics is normally employed as the industrial precursor of 6-APA and 7-ADCA.

The pathway from isopenicillin N to cephalosporins comprises several steps:[15] epimerization to penicillin N, which has a δ-(D-α-aminoadipyl) side chain, followed by ring expansion to desacetoxycephalosporin C, hydroxylation to desacetylcephalosporin C and then acylation to yield cephalosporin C, **7**. Since cephalosporin C, the normal product of fermentation of *Cephalosporium acremonium*, has a hydrophilic side chain, it is more difficult to isolate than penicillin G or penicillin V, and its production cost is estimated at $40-50/kg.[17]

There is no pathway in *C. acremonium* or in any other cephalosporin producing organism that allows the enzymatic conversion of cephalosporin C to 7-ACA or to a cephalosporin C analogue having a hydrophobic side chain. Despite extensive screening of the microbial kingdom, an acylase able to act effectively upon the δ-(D-α-aminoadipyl) moiety has not yet been disclosed. According to recent reports, however, this may soon change.[18]

The annual production of cephalosporin C is approximately 1000 tons, and 7-ACA, the precursor of the parenteral cephalosporins, has to

be prepared from the fermentation product by a chemical process,[19] at a cost of $250/kg.[17].

The economics of a biological process include lower energy and raw material (solvents and chemicals) costs, and low environmental impact because of the absence of toxic intermediates and waste products. Based on the data presented above, a catalytic antibody route from cephalosporin C to 7-ACA involving the phosphonamide **8** as an antibody hapten could be expected to reduce the cost of this bulk chemical by at least $100/kg or $100 million per annum. The intense interest in such a development is understandable.

1.5 Research goals

The objective of this research was to develop a synthesis of the phosphonamide **8** and related compounds and to become familiar with the syntheses and properties of phosphonamidates and phosphonamides in general.

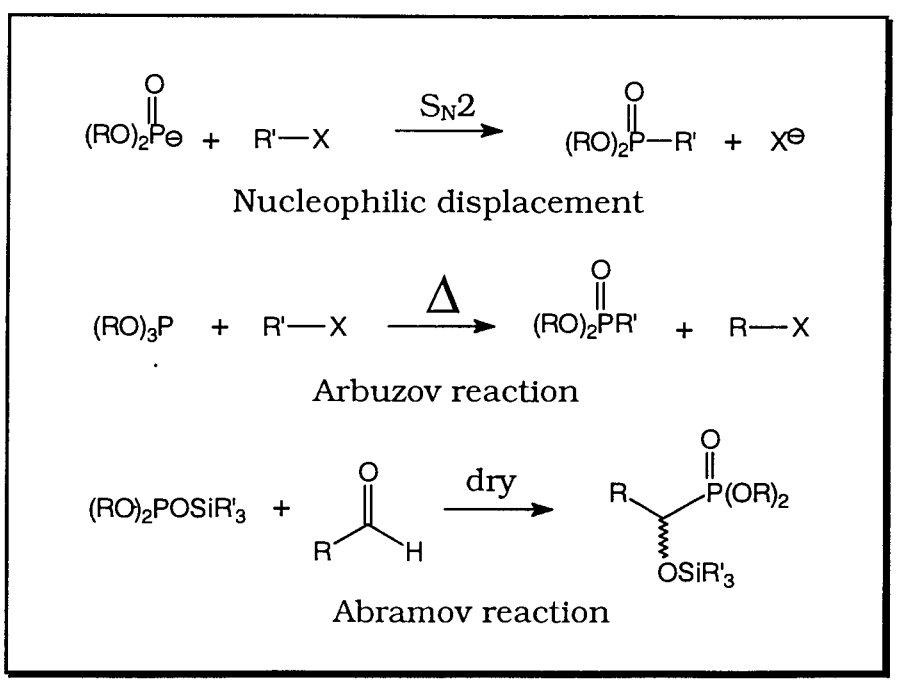
Chapter 2

2.1 Syntheses

The development of a synthetic route to **8** has required a study of methods to form phosphorous-carbon and phosphorous-nitrogen bonds.

2.1.1 Formation of phosphorous-carbon bonds

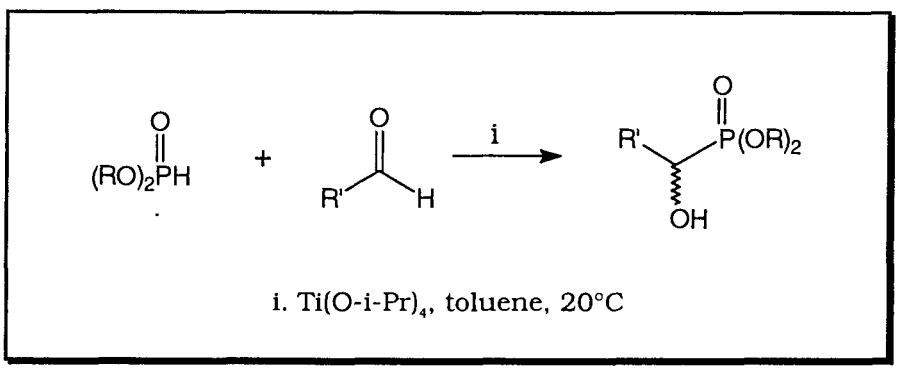
Along with the familiar nucleophilic displacement type of synthesis, the Michaelis-Arbuzov[20] and Abramov[21] reactions are two common methods employed for the formation of phosphorous-carbon bonds (Scheme 1).



Scheme 1

The forcing conditions of the Michaelis-Arbuzov reaction (high temperatures, long reaction times) make it less useful when the phosphite or alkyl halide contains sensitive functional groups.

On the other hand, the Abramov reaction typically involves the addition of a dialkylsilylphosphite to an aldehyde at room temperature.[22] Recent modifications[23] have led to the inclusion of the more stable hydrogen phosphonates as suitable reagents, and have increased the utility and scope of this reaction (Scheme 2).

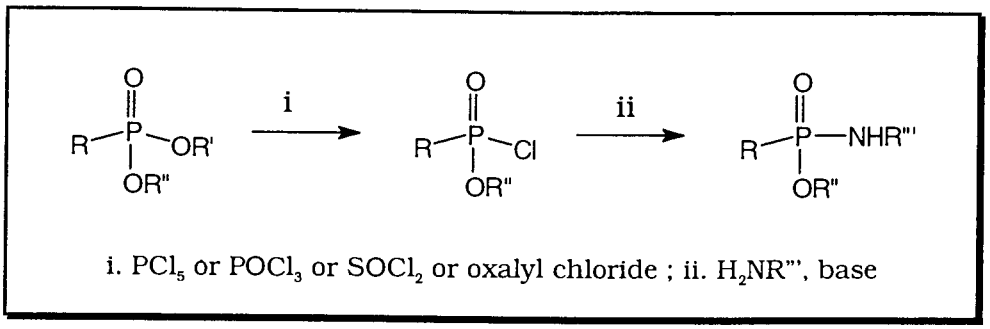


Scheme 2

2.1.2 Formation of phosphorous-nitrogen bonds

A major impetus for the study of compounds containing phosphorous-nitrogen bonds has been an interest in phosphonopeptides as isosteres of peptides. Some of this work has been attempted utilizing complex coupling methods.[24]

A particularly useful route to the phosphonamidates employs a phosphonochloridate, generated in various ways[25] (Scheme 3, R' = R" = alkyl). However, it is difficult to avoid overchlorination in step (I).



i. PCl_5 or $POCl_3$ or $SOCl_2$ or oxalyl chloride ; ii. $H_2NR"'$, base

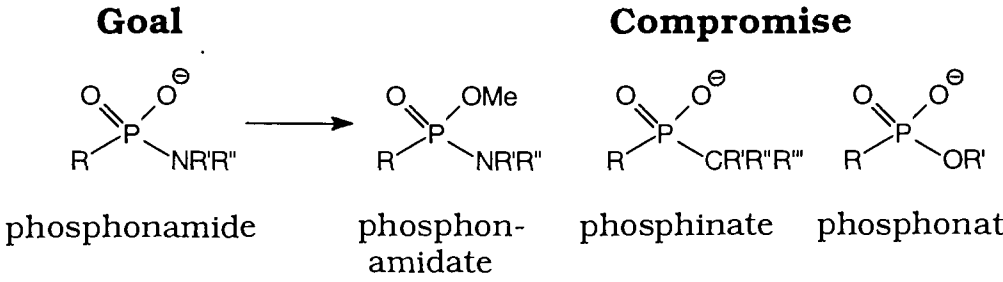
Scheme 3

Higher yields and cleaner reactions are observed when the monophosphonochloridate is generated from a mono rather than a dialkyl phosphonate[26] (R' = hydrogen, R" = alkyl).

2.1.3 Phosphonamidates and their deprotection

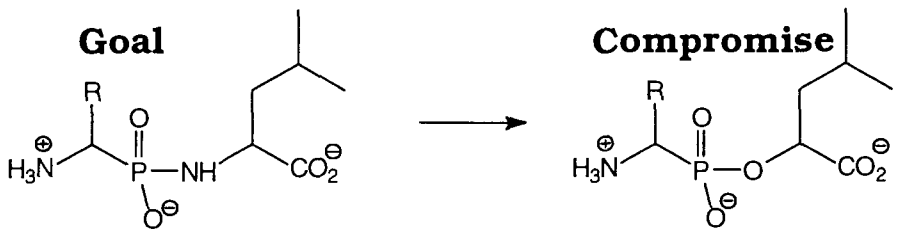
The usefulness of a given reaction sequence in a synthesis obviously depends on the yield of any final deprotection step. It is in the unmasking of phosphonamidates to phosphonamides that a major problem is encountered, namely, the great instability of the phosphorous-nitrogen bond of a phosphonamide under slightly acidic conditions.

W. J. Moree, G. A. van der Marel, J. H. van Boom, M. J. Liskamp, *Tetrahedron*, **49** 11055 (1993)



phosphonamide | phosphon-amidate | phosphinate | phosphonate

P. P. Giannousis, P. A. Bartlett, *J.Med.Chem.*, **30** 1603 (1987)



phosphonamide | phosphonate

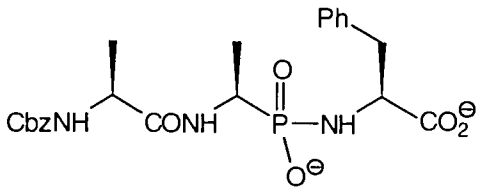
Scheme 4

Several research groups have been forced to modify their synthetic objectives to alleviate this problem (Scheme 4).

2.1.4 Aqueous stability of phosphonamides

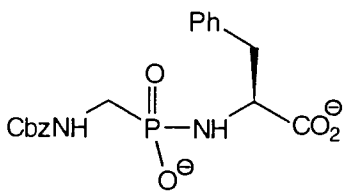
Several investigations, including our own, were undertaken to understand more completely the boundaries of phosphonamide stability in water. The great hydrolytic lability of phosphonamides is seen in the half-lives for hydrolysis shown in Figure 2.1.

J. E. Hanson, A. P. Kaplan, P. A. Bartlett, *Biochem.*, **28** 6294 (1989)



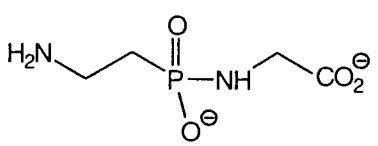
$t_{1/2}$ = 20 minutes
pH = 7.5
25° C

N. E. Jacobsen, P. A. Bartlett, *J.Am.Chem.Soc.*, **103** 654 (1981)



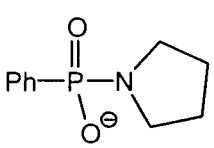
$t_{1/2}$ = 4 hours
pH = 6.2
21° C

K. Yamauchi, S. Ohtsuki, M. Kinoshita, *J.Org.Chem.*, **49** 1158 (1984)

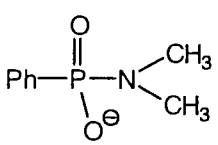


$t_{1/2}$ = 4 hours
pH = 7
25° C

J. Rahil, P. Haake, *J.Am.Chem.Soc.*, **103** 1723 (1981)



$t_{1/2}$ = 50 minutes
pH = 6
30° C



$t_{1/2}$ = 15 minutes
pH = 6
30° C

Figure 2.1. Half-lives of phosphonamides in aqueous solution

In the detailed study of Haake and Rahil[27] the kinetics of hydrolysis were consistent with an initial protonation at the phosphonamide nitrogen, followed by attack of water (Figure 2.2).[28]

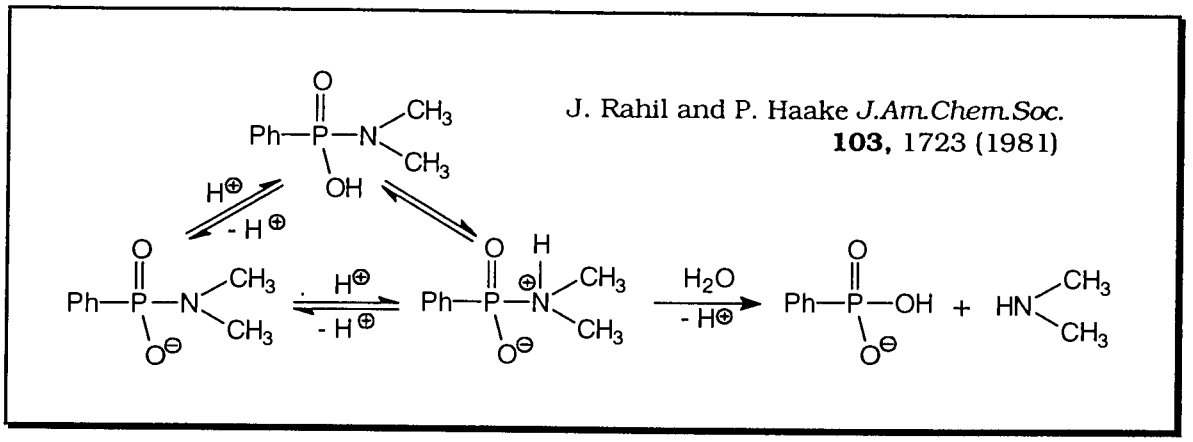


Figure 2.2. A mechanism for acid catalyzed hydrolysis of phosphonamides

2.1.5 Protecting groups for phosphonamides

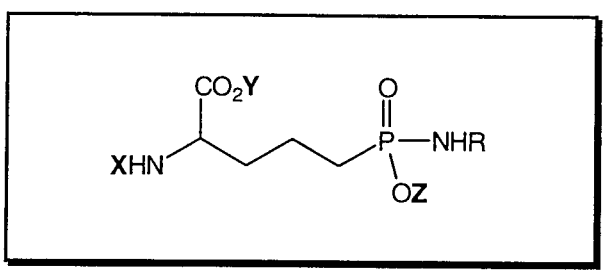
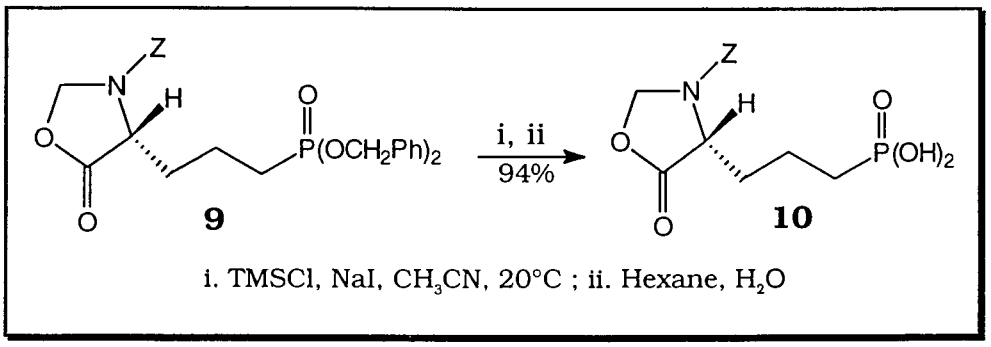


Figure 2.3. A generally protected phosphonamide

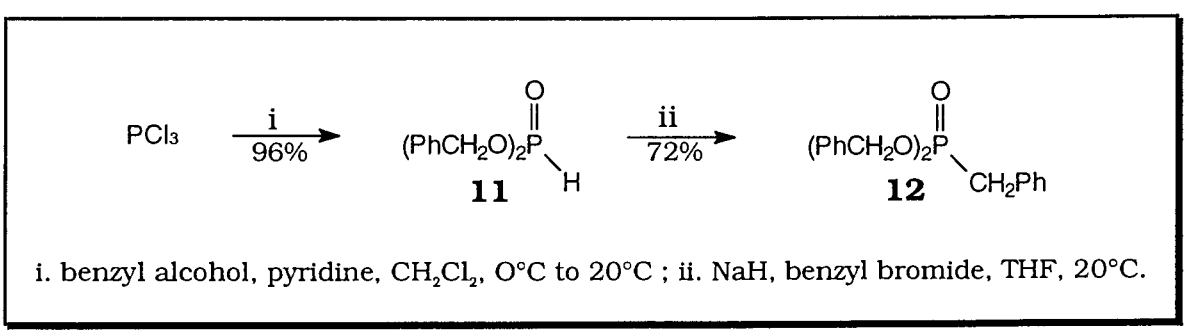
For the generalized phosphonamide shown in Figure 2.3 it is clear from the previous section that acidic removal of protecting groups is precluded. A possible option for neutral cleavage of the phosphorous ester involved using a trimethylsilyl halide (Scheme 5),[29] but this has been linked to phosphorous-nitrogen cleavage.[30] Of the remaining options, the best appeared to involve the use of benzyl esters combined with a mild deprotection step.



Scheme 5

2.1.6 Synthesis of a dibenzyl phosphonate

The dibenzyl hydrogen phosphonate **11** was produced by the addition of benzyl alcohol to phosphorous trichloride in methylene chloride containing pyridine at 0° C (Scheme 6). Stirring the mixture at room temperature for 24 hours produced a near quantitative yield of **11**.

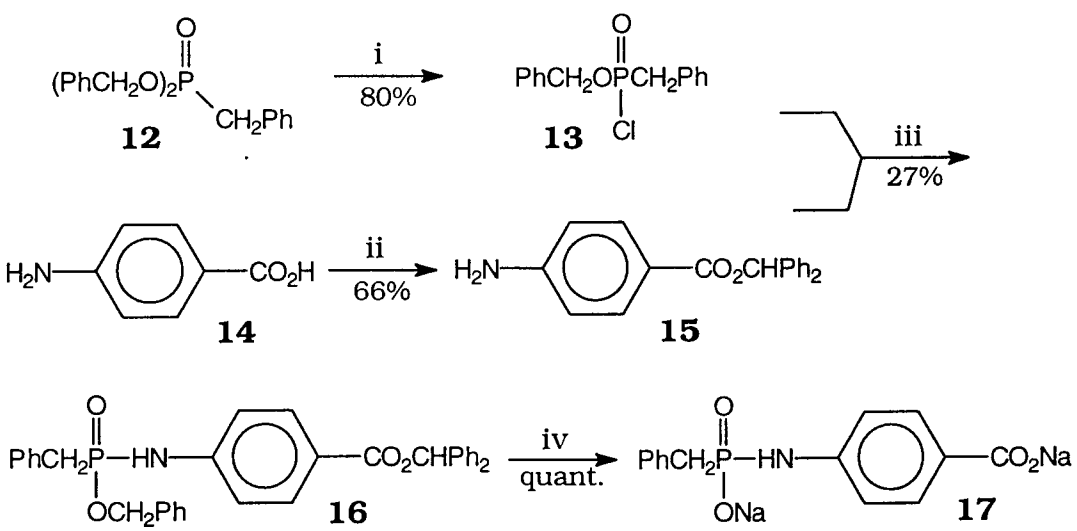


Scheme 6

Several attempts to prepare **12** via a Michaelis-Becker reaction[31] with sodium hydride and benzyl chloride were unsuccessful. Eventually the problem was solved[32] by slow addition of the hydrogen phosphonate to a suspension of sodium hydride in tetrahydrofuran followed by addition of an alkyl bromide. These conditions produced **12** in 72% yield.

2.1.7 Synthesis of a model phosphonamide

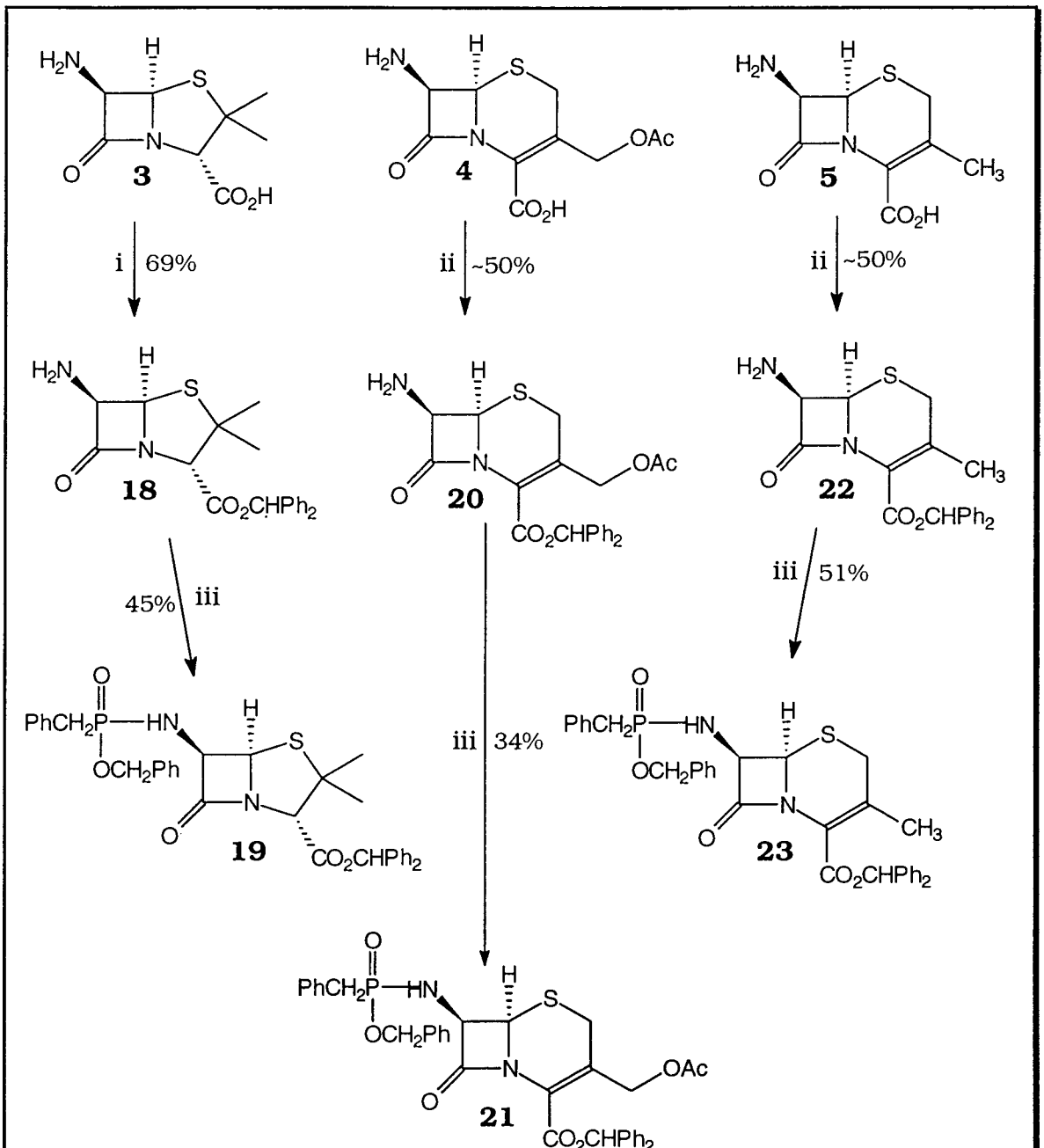
The synthesis of **17** began with the protection of p-aminobenzoic acid, **14**, with diphenyldiazomethane in methylene chloride, to give the amino ester **15** (Scheme 7), and the treatment of the phosphonate **12** with a slight excess of oxalyl chloride and a catalytic amount of dimethylformamide at 50° C. The conversion to **13** was followed by ^{1}H NMR, and the disappearance of **12** and concurrent appearance of benzyl chloride and **13** indicated the extent of reaction. When the conversion of **12** was complete, this mixture was combined with **15** in methylene chloride in the presence of pyridine to form **16**. Hydrogenolysis[33] in the presence of sodium bicarbonate then afforded **17** quantitatively.



i. oxalyl chloride, cat. DMF, 50°C ; ii. diphenyldiazomethane, CH_2Cl_2, 20°C ; iii. pyridine, CH_2Cl_2, 20°C ; iv. $NaHCO_3$, Pd/C, H_2, H_2O, THF.

Scheme 7

2.1.8 Coupling of a benzyl phosphonate to the penicillin and cephalosporin nuclei



i. a. diphenyldiazomethane, refluxing CH_3CN/CH_2Cl_2, 24 hrs. b. p-TsOH•H_2O, diethyl ether, acetone ; ii. diphenyldiazomethane, refluxing CH_3CN/CH_2Cl_2, 24 hrs; iii. **13**, pyridine, CH_2Cl_2, 20°C

Scheme 8

In addition to providing novel compounds with potential antibacterial activity, the phosphonamidate/β-lactam system allows for a more complete exploration of deprotection conditions. The base-sensitive β-lactam ring must now be considered (Figure 2.4), as well as the acid sensitive phosphorous-nitrogen bond.

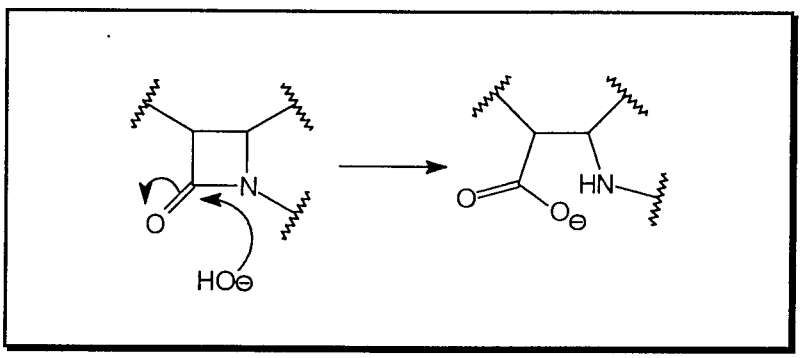
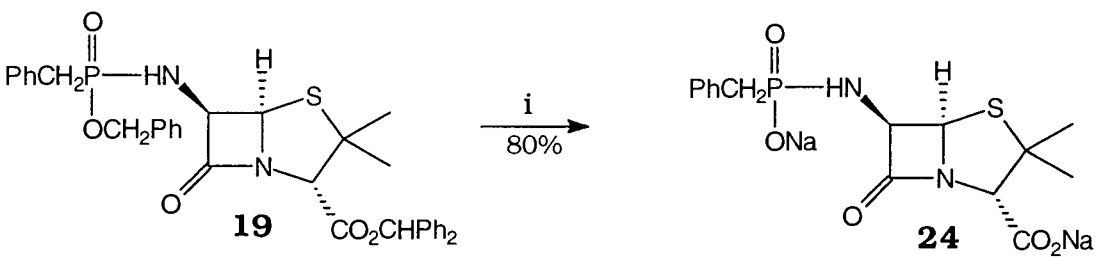


Figure 2.4. β-lactam opening due to attack of hydroxyl

6-Aminopenicillanic acid (6-APA, **3**), 7-aminocephalosporanic acid (7-ACA, **4**), and 7-aminodesacetoxycephalosporanic acid (7-ADCA, **5**) were esterified with diphenyldiazomethane in methylene chloride and acetonitrile over 24 hours (Scheme 8). The penicillin ester **18** was isolated as the crystalline p-toluenesulfonic acid salt which could be stored until required. The cephalosporin esters **20** and **22** were not isolated as easily and were purified by column chromatography immediately before coupling to benzyl phosphonochloridate **13**.

The free amine of **18** was generated by neutralization with sodium bicarbonate and extraction into methylene chloride, and was coupled to **13** in the presence of pyridine. Each of the phosphonamidates **19**, **21**

and **23** was isolated as a mixture of diastereomers epimeric at phosphorous and, in the case of **19**, separable by chromatography. Hydrogenolysis of **19** gave the disodium salt **24** (Scheme 9).

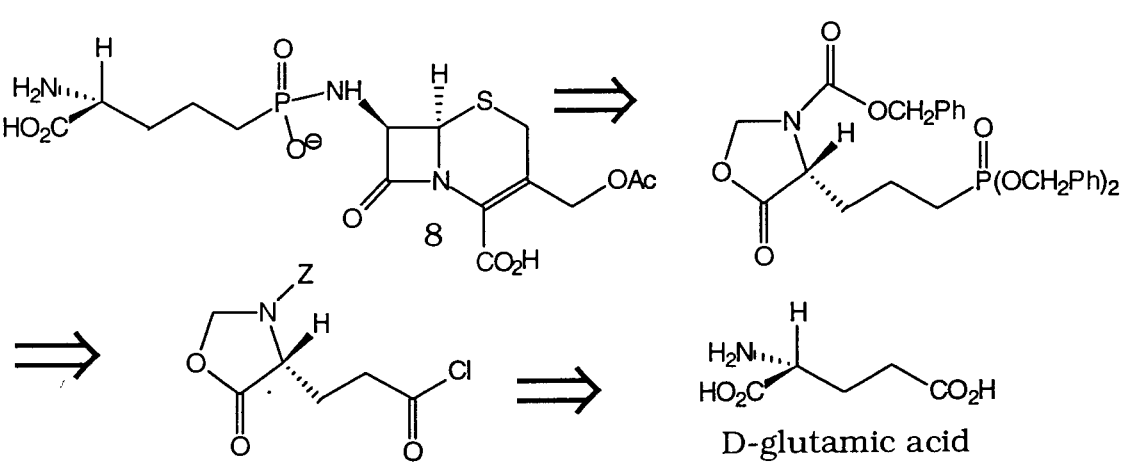


i. $NaHCO_3$, Pd/C, H_2, H_2O, THF.

Scheme 9

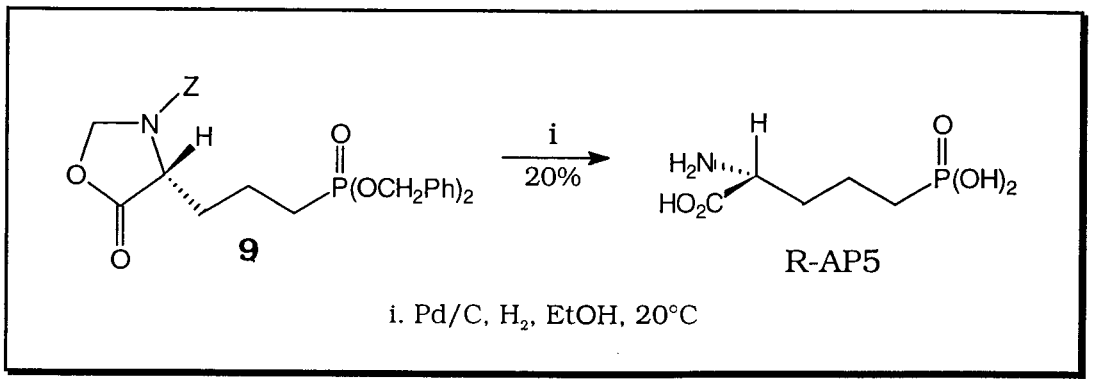
2.1.9 Synthesis of the R-2-amino-5-phosphonopentanoic acid [R-AP5] side chain

A possible starting point for the synthesis of the phosphonamide side chain of **8** is D-glutamic acid (Scheme 10).



Scheme 10

The validity of this path was realized by the successful synthesis of the potent neurochemical R-2-amino-5-phosphonopentanoic acid (R-AP5)[34] by hydrogenolysis of the intermediate **9** (Scheme 11).

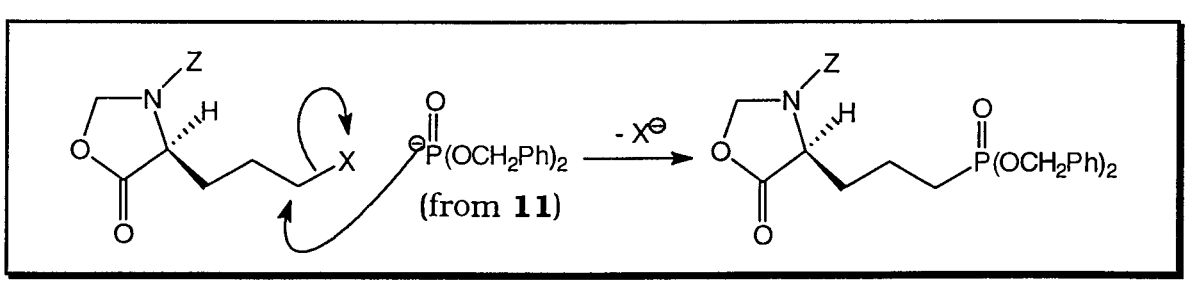


Scheme 11

The earliest syntheses of AP5 in racemic form are those of Evans,[35] Matoba[36] and Ornstein.[37]

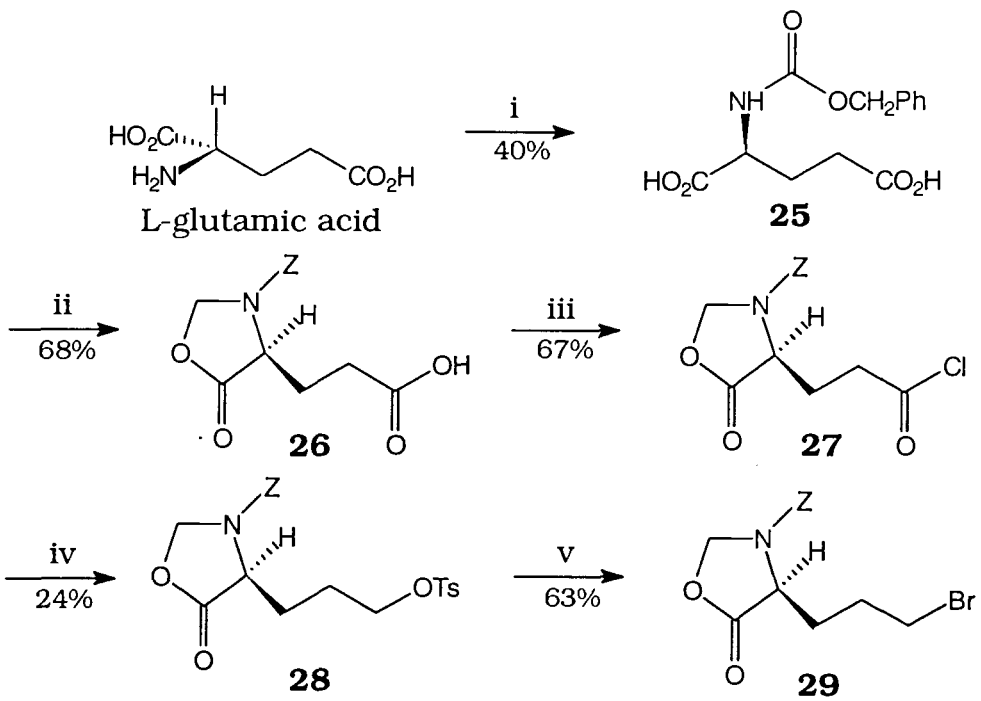
The first asymmetric synthesis of R-AP5, reported by Schollkopf, utilizes the bislactim ether method.[38] Ornstein[39] followed with Dellaria's oxazinone glycine approach.[40] There are also several other contemporary routes to R-AP5.[41]

The initial strategy to synthesize the side chain of **8** was similar to Ornstein's earlier work on racemic AP5.



Scheme 12

It was proposed that the nucleophilic displacement of a leaving group by a phosphonate anion would produce **9** directly (Scheme 12), by reasoning of our success in the synthesis of **12** with the anion of **11**. Note that L-glutamic acid was employed in the developmental stages of the project, as it is more readily available than the D form.



i. $NaHCO_3$, benzyl chloroformate, H_2O, 20°C; ii. paraformaldehyde, p-TsOH, toluene, reflux ; iii. oxalyl chloride, cat. DMF, toluene, 20°C to 50°C ; iv. $NaBH_4$,DME,H_2O, -8°C; Pyridine, TsCl, $CHCl_3$, 0°C; v. Tetrabutylammonium bromide, CH_2Cl_2, 35°C.

Scheme 13

As shown in Scheme 13, L-glutamic acid was dissolved in water using excess sodium bicarbonate, and acylated with benzyl chloroformate to give the protected diacid **25**. This was converted to the

oxazolidinone **26**[42] by refluxing in toluene with paraformaldehyde and a catalytic amount of p-toluenesulfonic acid, using a Dean-Stark trap to effect azeotropic removal of water. The yellow, crystalline acid chloride **27** was generated by gentle heating of the acid with excess oxalyl chloride and a drop of dimethylformamide in toluene. The reduction of the acid chloride and tosylation of the resulting alcohol to **28** was accomplished with sodium borohydride in water and dimethoxyethane, followed by addition of tosyl chloride in chloroform. The bromide **29** was then generated by treatment of **28** with tetrabutylammonium bromide in refluxing methylene chloride.

All attempts to couple **11** with **29** failed. The isolated product (Figure 2.5) indicated loss of one benzyloxy group from the phosphonate,

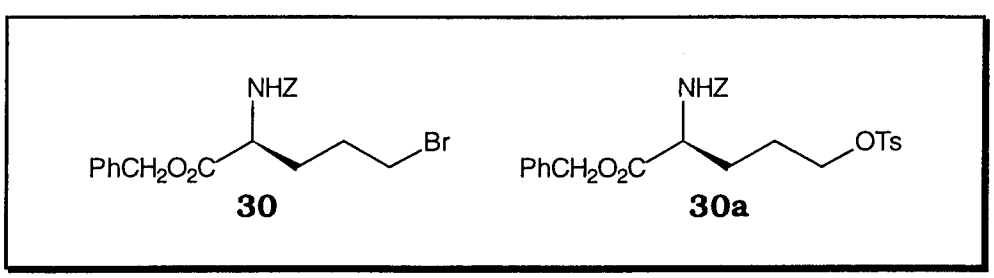


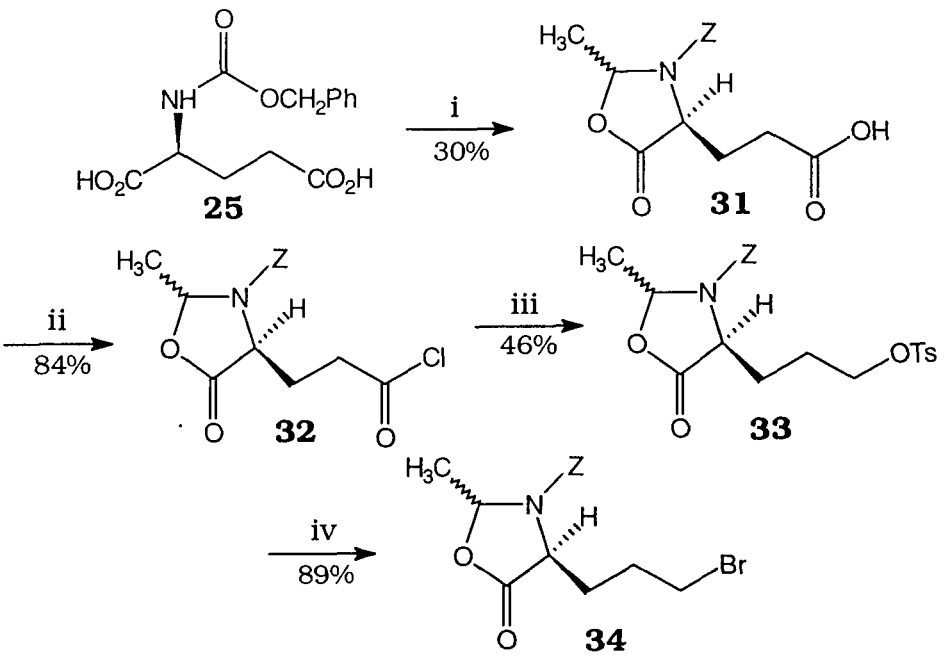
Figure 2.5. Structures of **30** and **30a**

and showed complex ^{1}H NMR spectra, eventually interpreted in terms of structure **30** (See Section 3.1, p. 43).

The reaction was also carried out on the tosylate **28**, but resulted in a very similar compound **30a**. In both cases it appeared that the leaving groups were not affected at all.

Because the oxazolidinone was being destroyed in the process, an extra alkyl group was introduced into the ring to increase its stability (Scheme 14).

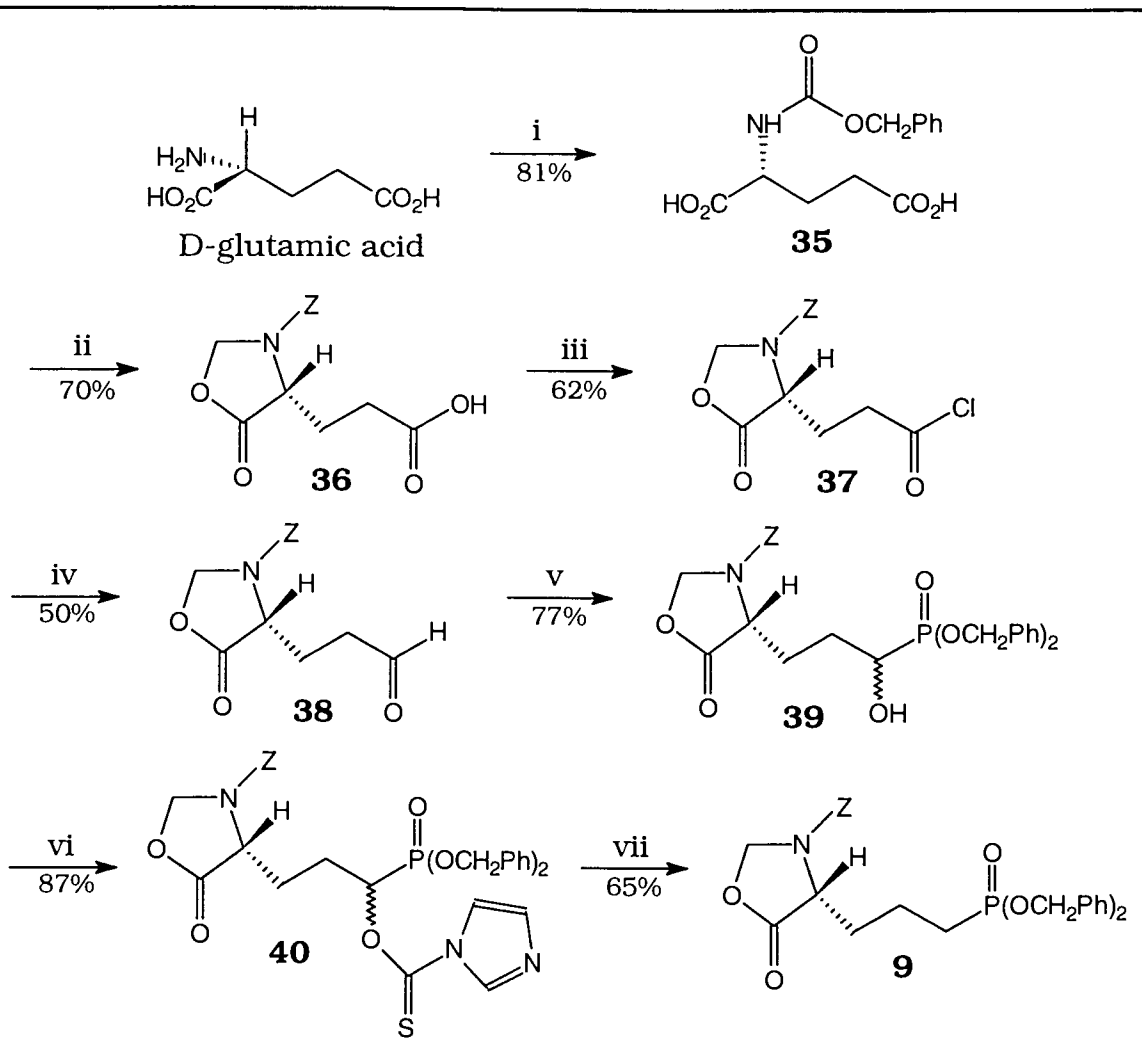
The procedures involved in the synthesis of **34** were identical to those in Scheme 13, except that the initial ring formation employed acetaldehyde in place of paraformaldehyde.



i. acetaldehyde, p-TsOH,toluene, reflux ; ii. oxalyl chloride, cat. DMF, toluene, 20°C to 50°C; iii. $NaBH_4$,DME,H_2O, -8°C; Pyridine, TsCl, $CHCl_3$, 0°C; iv.Tetrabutylammonium bromide, CH_2Cl_2, 35°C.

Scheme 14

This strategy did not affect the outcome of the coupling reaction, and the product **30** was again isolated.



i. $NaHCO_3$, benzyl chloroformate, H_2O, 20°C; ii. paraformaldehyde, p-TsOH, toluene, reflux ; iii. oxalyl chloride, cat. DMF, toluene, 20°C to 50°C ; iv. $LiAlH(O\text{-}t\text{-}Bu)_3$, THF, -78°C ; v. dibenzylphosphonite, $Ti(O\text{-}i\text{-}Pr)_4$, toluene, 20°C ; vi. thiocarbonyldiimidazole, 1,2-dichloroethane, 60°C ; vii. Bu_3SnH, benzene, reflux.

Scheme 15

The required intermediate **9** was eventually synthesized by the route summarized in Scheme 15. D-Glutamic acid was transformed to the acid chloride **37** as already outlined in Scheme 13. The acid chloride was reduced to the aldehyde **38** using lithium tri-t-butoxyaluminum hydride[43] in tetrahydrofuran at -78°C.

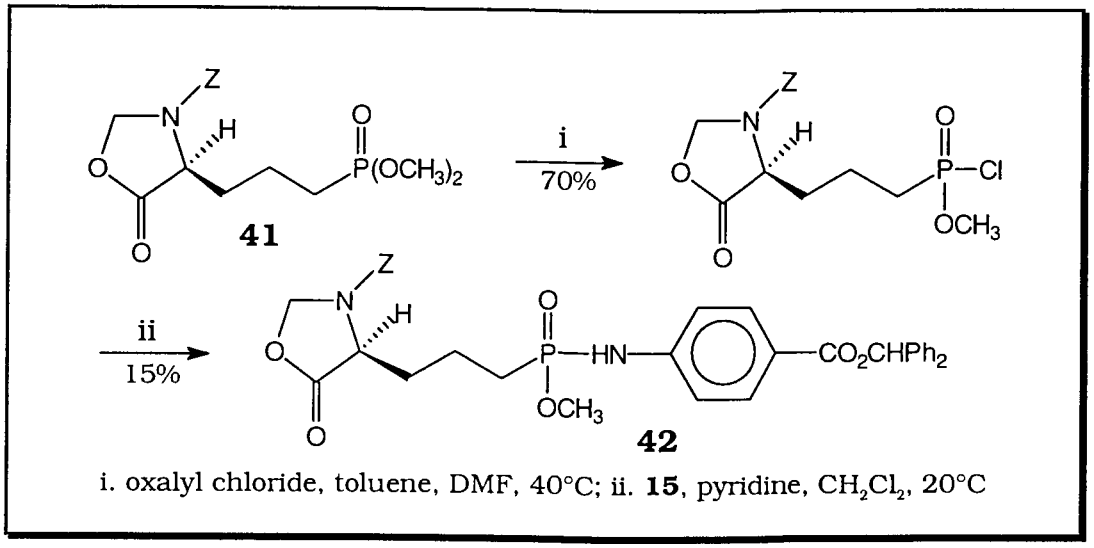
In this first route to **9**, the aldehyde **38** was reacted with a silylphosphite under rigorously dry conditions to yield first a siloxyphosphonate (as in Scheme 1) and then, after acid hydrolysis, the hydroxy phosphonate **39**.[44] However, the lability of the silylphosphite, moderate yields and added hydrolysis step made this sequence unattractive. A modified Abramov reaction was therefore employed, and this solved each of the problems.[23] The aldehyde was reacted with the dibenzyl hydrogen phosphonate **11** in the presence of titanium tetra-i-propoxide in toluene to give **39** directly in good yield.

The final steps to the phosphonate **9** involved the removal of the hydroxyl function in **39**.[44] The alcohol was first transformed to the thiocarbonylimidazole **40** with thiocarbonyldiimidazole in dichloroethane. This group was then removed by the method of Barton and McCombie[45] using tri-n-butyl tin hydride in refluxing benzene.

2.1.10 Synthesis of the phosphonamidate hapten

The phosphonamidate **42** was synthesized from **41**[50] and **15** in order to test the practicality of treating an oxazolidinone phosphonate with the standard coupling conditions developed earlier (Scheme 16).

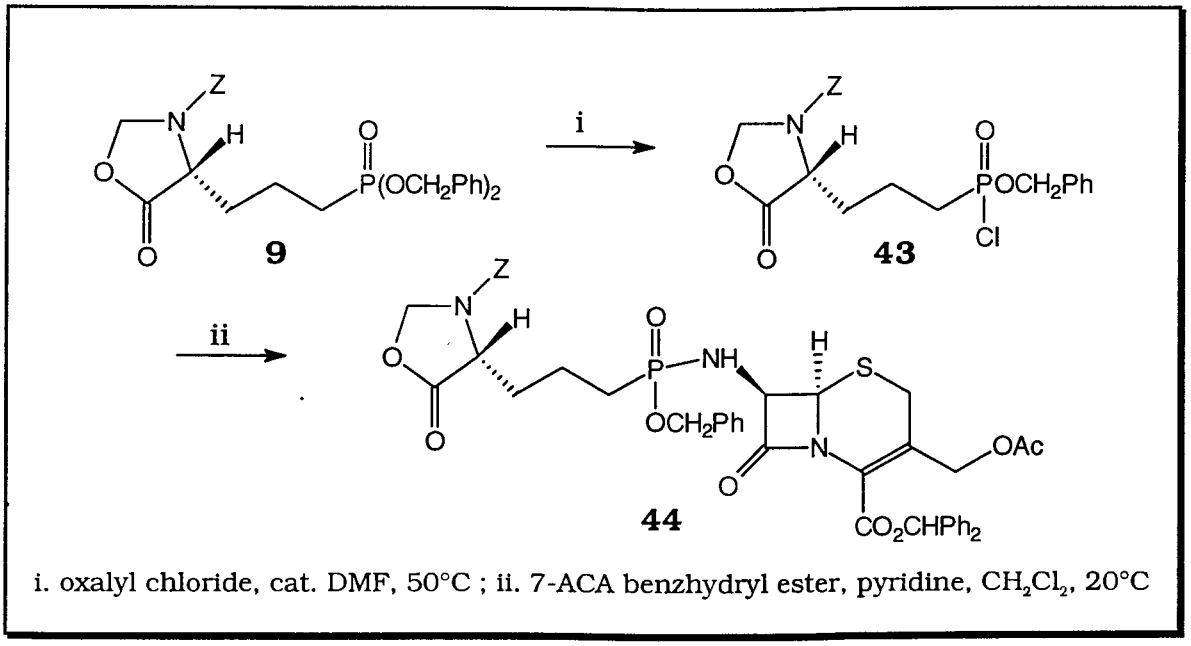
The success of this route suggested that coupling of the phosphonate **9** to the amino group of 7-ACA should be preceded by chlorodebenzylation of the phosphonate diester (Scheme 17).



i. oxalyl chloride, toluene, DMF, 40°C; ii. **15**, pyridine, CH_2Cl_2, 20°C

Scheme 16

The phosphonochloridate **43** was treated with 2.5 equivalents of the 7-ACA benzhydryl ester in methylene chloride. Following work-up, column chromatography revealed the presence of the two diastereomers of **44**, which were partially resolved.



i. oxalyl chloride, cat. DMF, 50°C ; ii. 7-ACA benzhydryl ester, pyridine, CH_2Cl_2, 20°C

Scheme 17

2.2 Properties of phosphonates, phosphonamidates and phosphonamides

2.2.1 Kinetics of hydrolysis

There is much experimental evidence of the acid lability of the phosphorous-nitrogen bond in phosphonamides.[46] The result of our study on the hydrolysis of **17** is presented below.

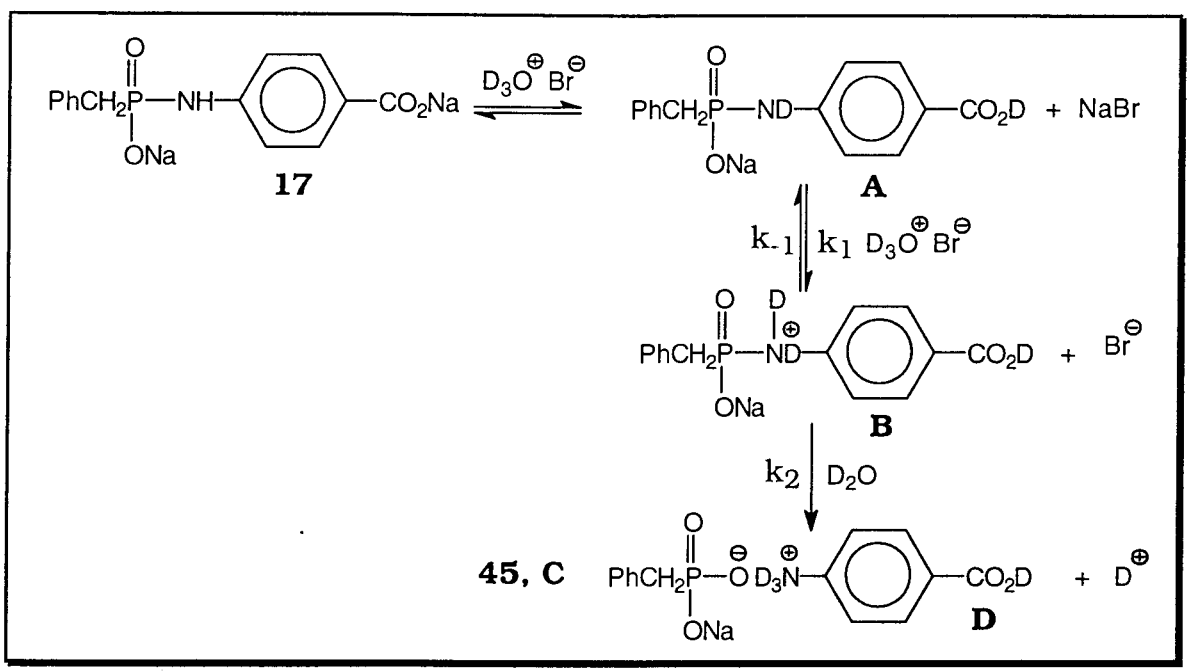


Figure 2.6. Proposed mechanism for the hydrolysis of **17**

The disodium salt **17** was titrated with 0.10 M DBr (Figure 2.7). The inflection point at one equivalent of acid corresponded to 300 μL of DBr, and indicated full deuteration of the carboxylic acid **A** (Figure 2.6). Addition of a further 0.3 equivalents of acid resulted in the precipitation of a white solid.

The estimated pKa value[47] for the carboxylic function is 4.8 and is very close to the value observed from the titration curve.

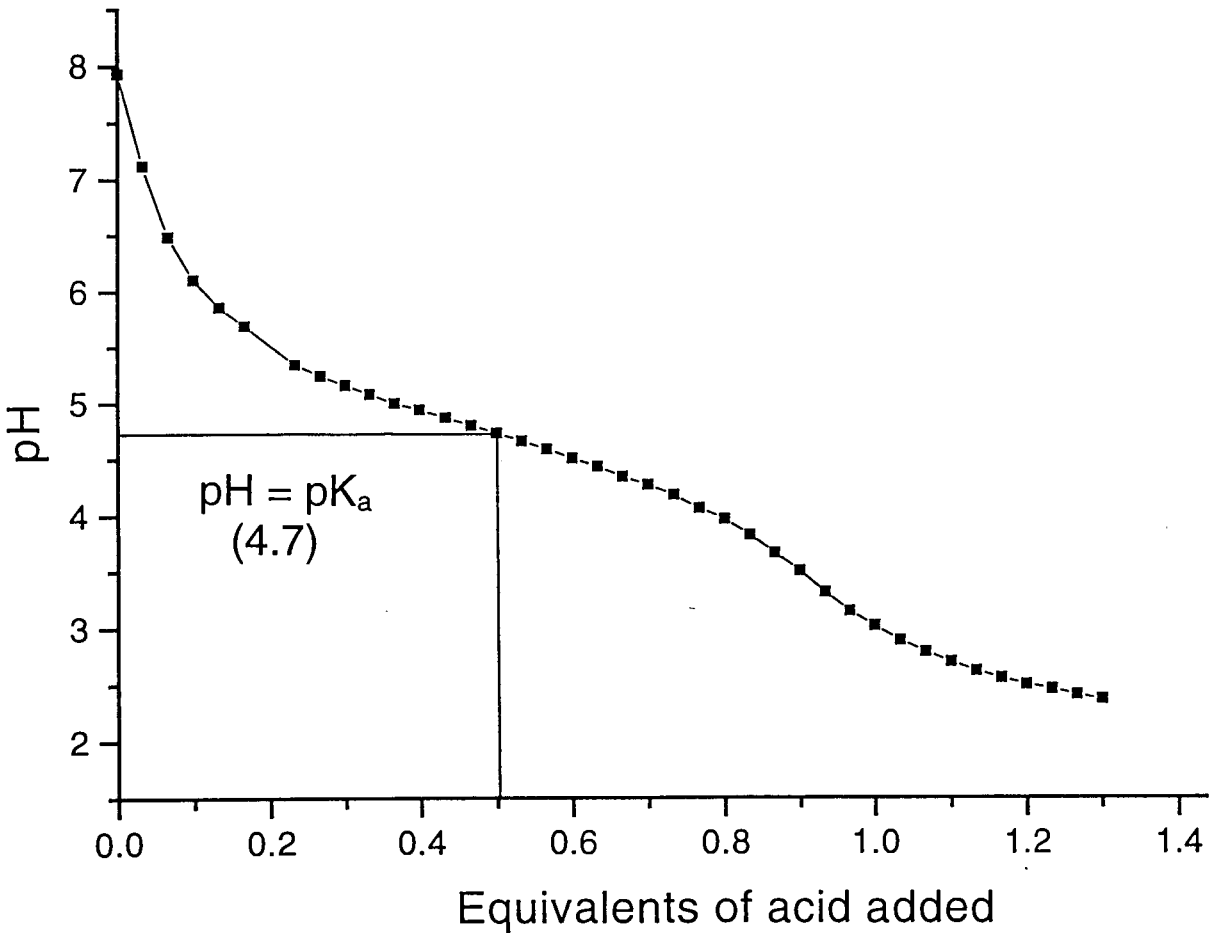


Figure 2.7. Titration curve of **17**

Rate calculations were performed assuming that (i) **A** and **B** establish an equilibrium rapidly; (ii) the rate determining step is the irreversible hydrolysis of the phosphorous-nitrogen bond; (iii) the pH of the reaction medium does not change appreciably during the hydrolysis. These assumptions are justified by the results of Rahill and Haake.[27]

Data were compiled on identical samples of **17** in D_2O using 1.2 equivalents of DBr. All reactions were run in NMR tubes at the temperatures specified in Table 2.1, set and monitored by a Bruker variable temperature control unit. Rates were determined by following the disappearance of the methylene doublet of **A** and **B**, and the appearance of the methylene doublet of **C** (Figure 2.8).

The observed rate constant value k_{obs} was determined using the first-order rate equation (1), where the terms in brackets are the

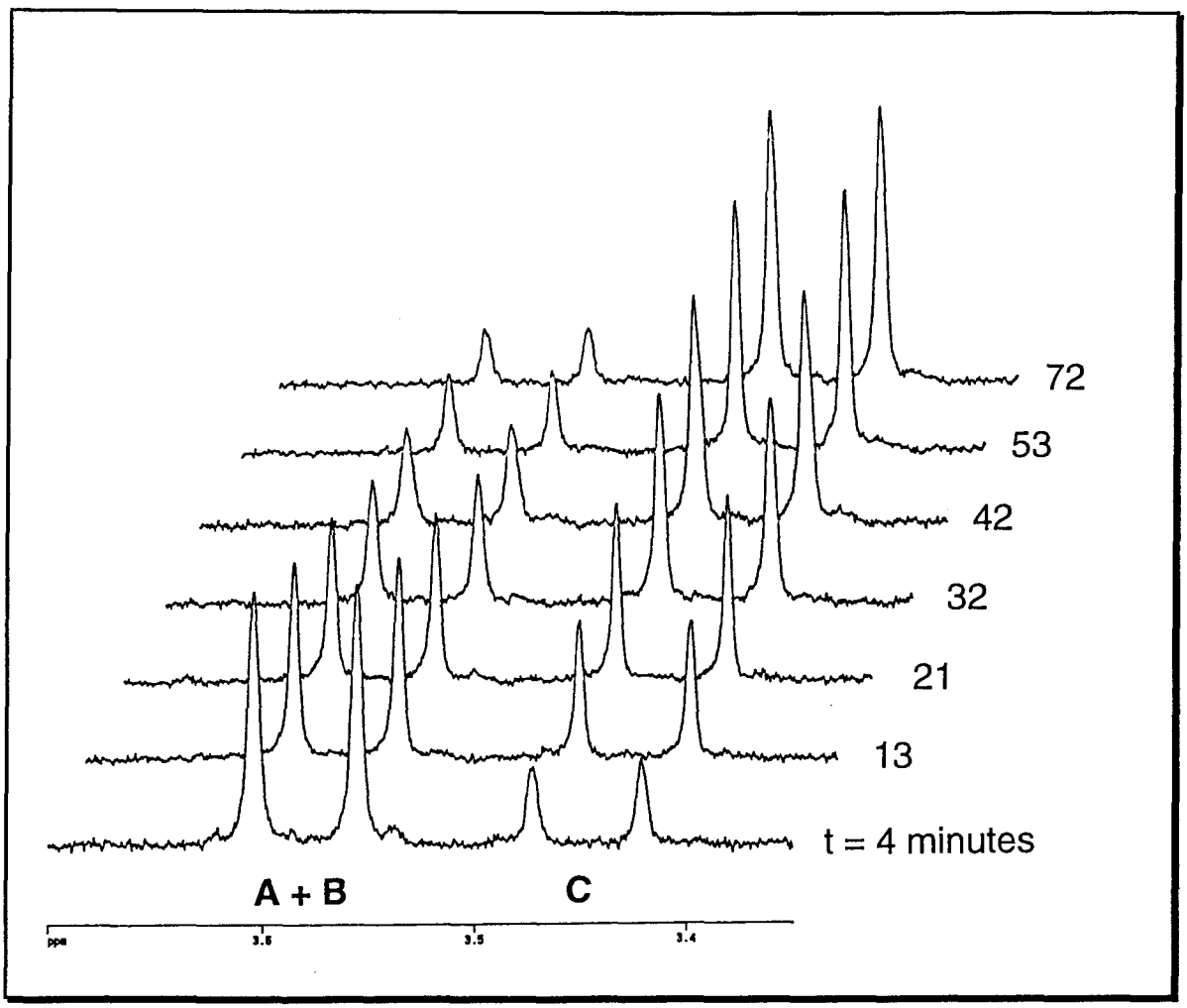


Figure 2.8 ^1H NMR signals showing decreasing **A**,**B** and increasing **C** at 60°C

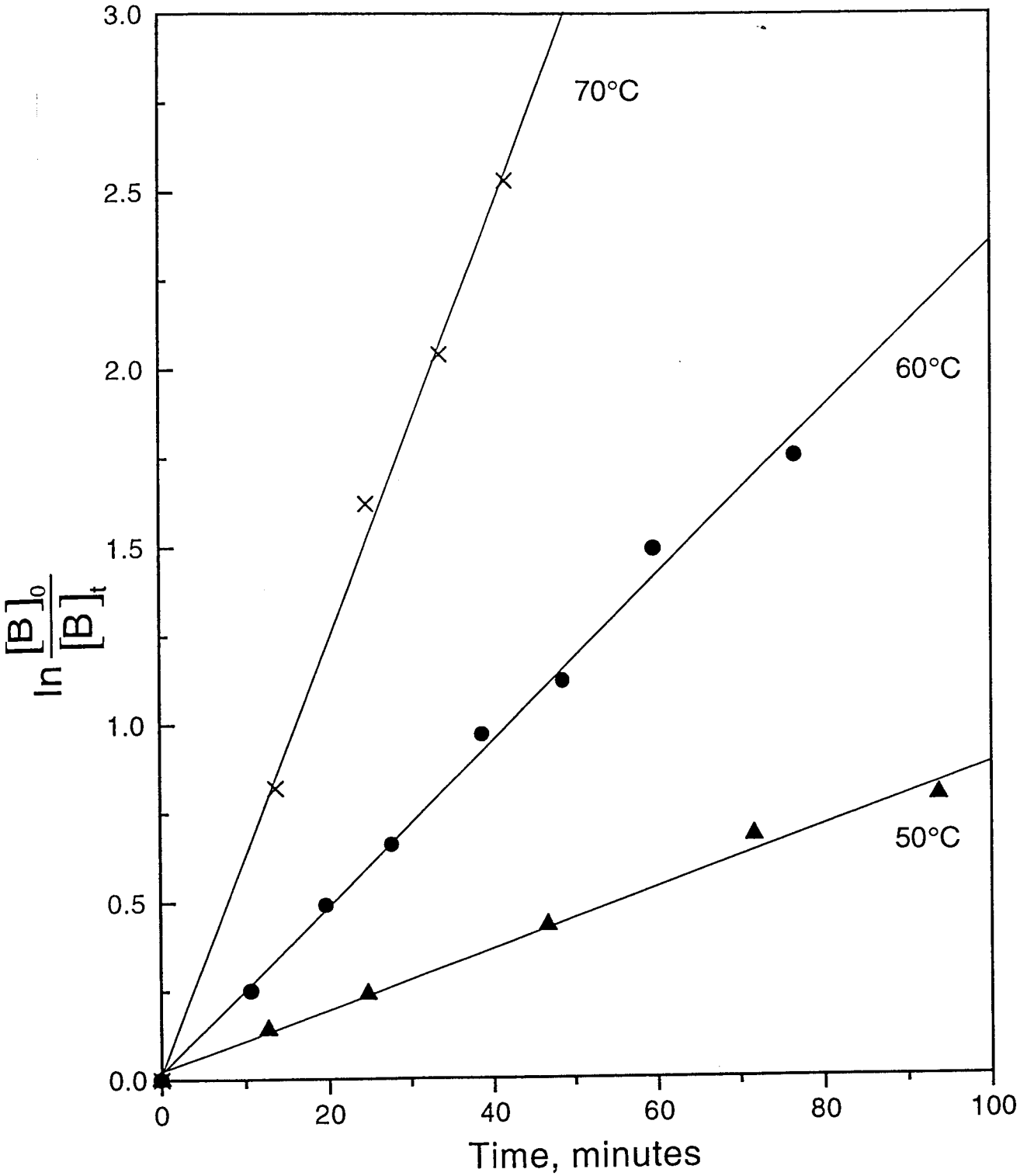


Figure 2.9. Plots of $\ln\left(\frac{[B]_0}{[B]_t}\right)$ versus time at 70°C, 60°C, 50°C

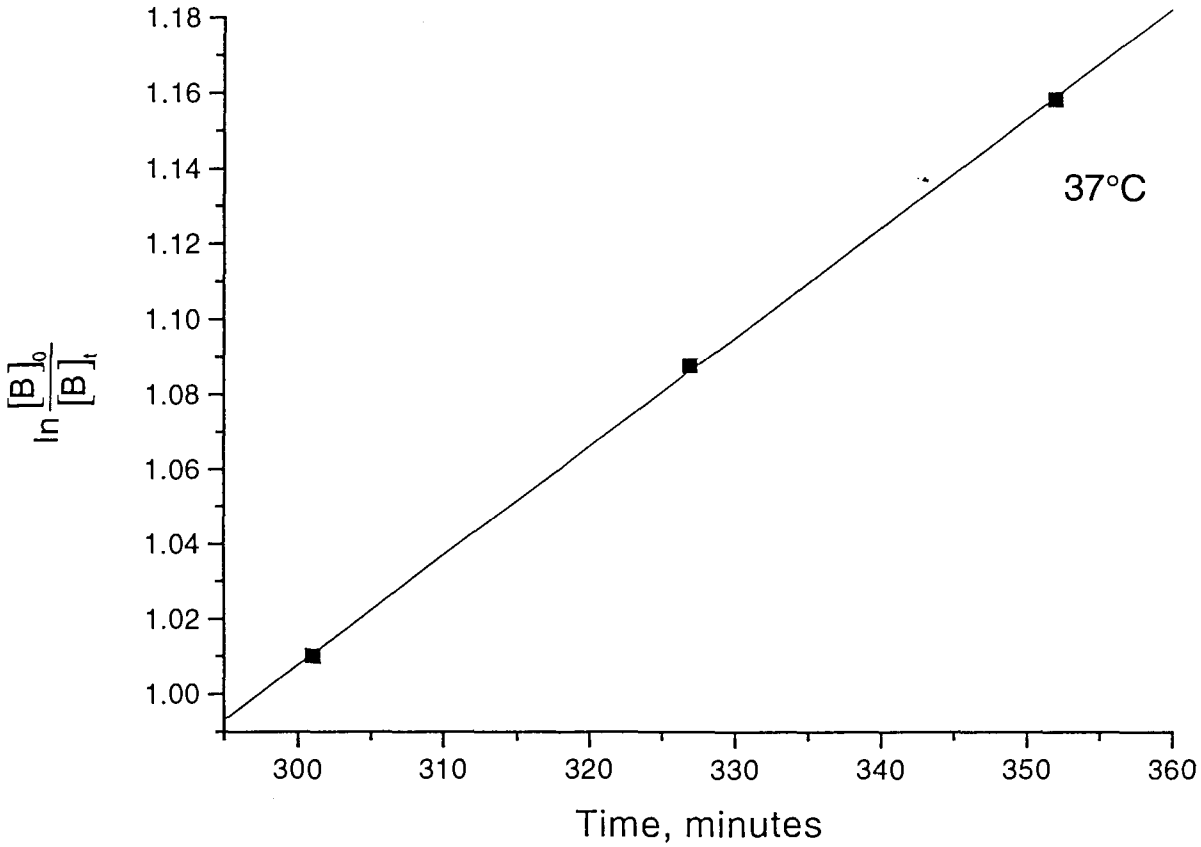


Figure 2.10. Plot of $\ln\left([\mathbf{B}]_0/[\mathbf{B}]_t\right)$ versus time at 37°C

Temperature, °C	Rate constant k_{obs}, s^{-1}
37.0 ± 0.2	(4.86 ± 0.17) x 10^{-5}
50.0 ± 0.2	(1.43 ± 0.13) x 10^{-4}
60.0 ± 0.2	(3.90 ± 0.24) x 10^{-4}
70.0 ± 0.2	(1.01 ± 0.07) x 10^{-3}

Table 2.1. Observed rate constants at several temperatures

concentrations (or associated areas under ^{1}H NMR peaks) of the phosphonamides **A** and **B** at time equal to **t** or zero.

$$\ln \frac{[\mathbf{B}]_0}{[\mathbf{B}]_t} = k_{obs} t \qquad (1)$$

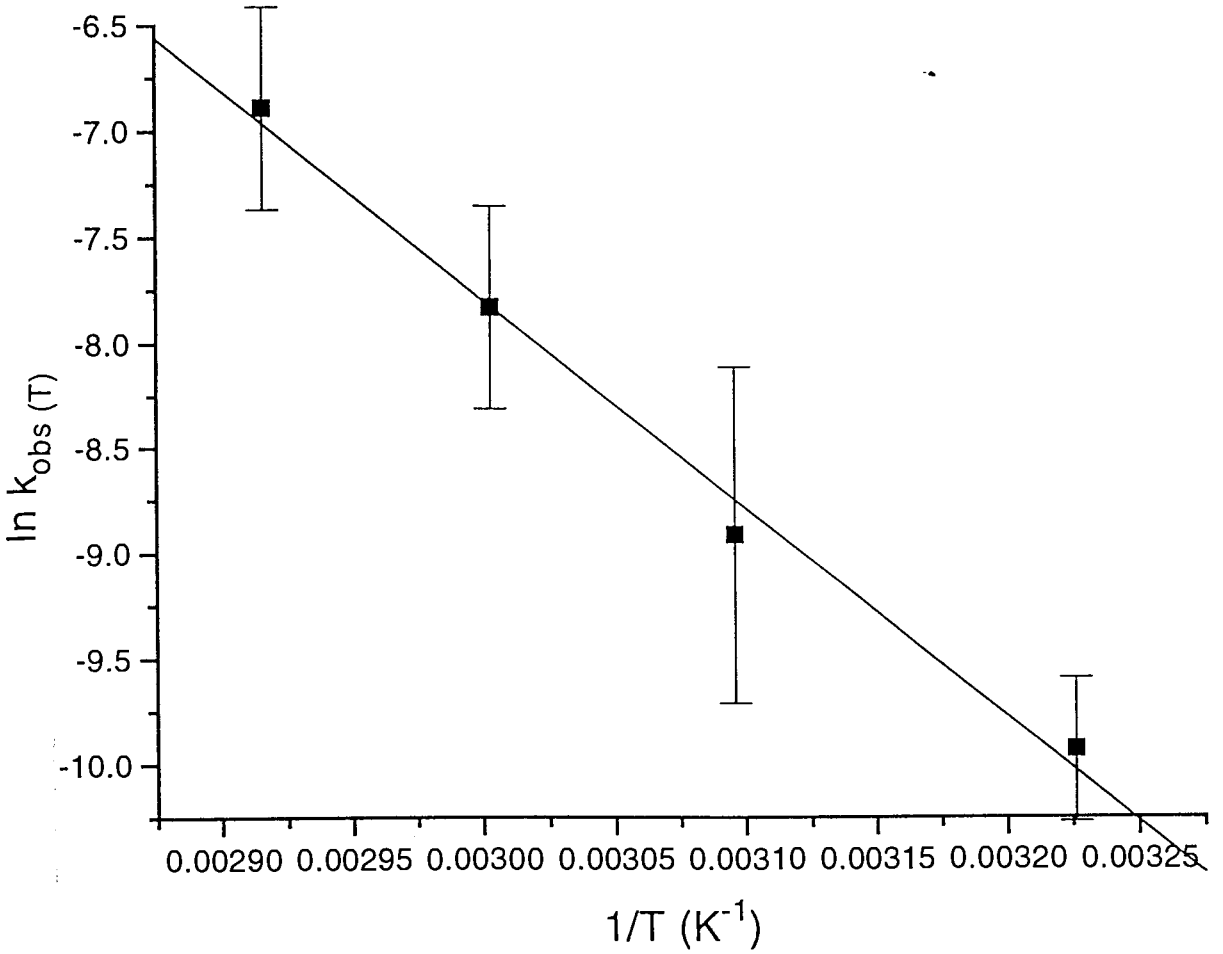


Figure 2.11. Plot of ln $k_{obs(T)}$ versus T^{-1} for the acid catalyzed hydrolysis of **17**

For the four temperatures studied the plots of ln versus time are linear, yielding the observed rate constants for hydrolysis in D_2O below pH 3.

The activation energy for hydrolysis was calculated using a standard Arrhenius plot, equation (2) and Figure 2.11, and found to be 88 ± 4 kJ/mol in D_2O.

$$\ln k_{obs(T)} = {}^{-\Delta E_{act}}\!/_{RT} + \ln A \qquad (2)$$

2.2.2 ^1H NMR investigations

The complexity of **19** and **44** (Figure 2.12) necessitated the use of 400 and 600 MHz NMR experiments to confirm the structures, to study various conformational processes and to carry out the aforementioned kinetic studies.

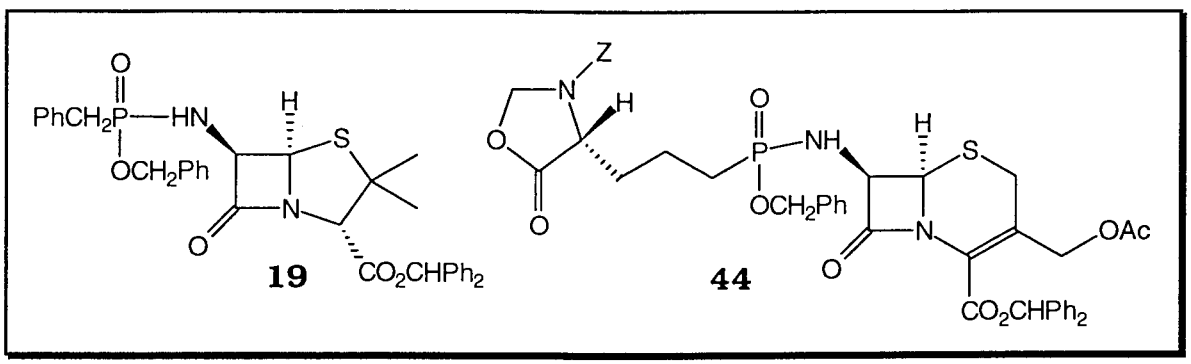


Figure 2.12. Structures of **19** and **44**

2.2.2.1 Low temperature conformation study

During the synthesis of **9** it became apparent that coupling of the aldehyde **38** to the phosphonate **11** led to severe broadening of the oxazolidinone ^1H NMR signals (Figure 2.13). This was believed to be due to a dynamic conformational effect.[48] This hypothesis was confirmed by low temperature ^1H NMR examination of **9** (Figures 2.14 and 2.15).

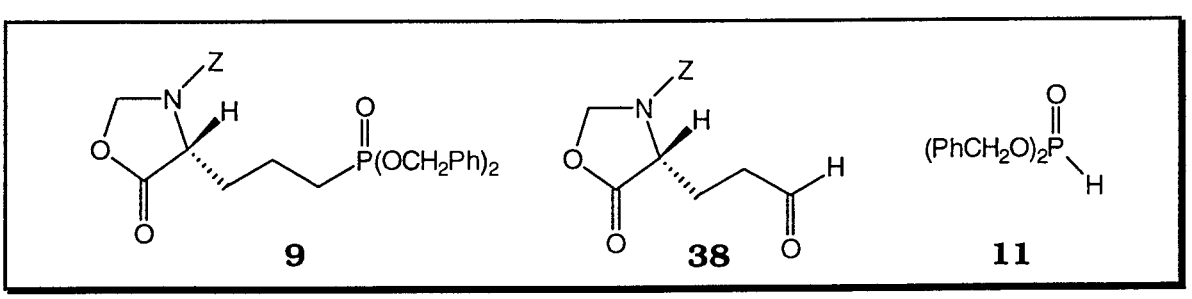


Figure 2.13. Structures of **9**, **38** and **11**

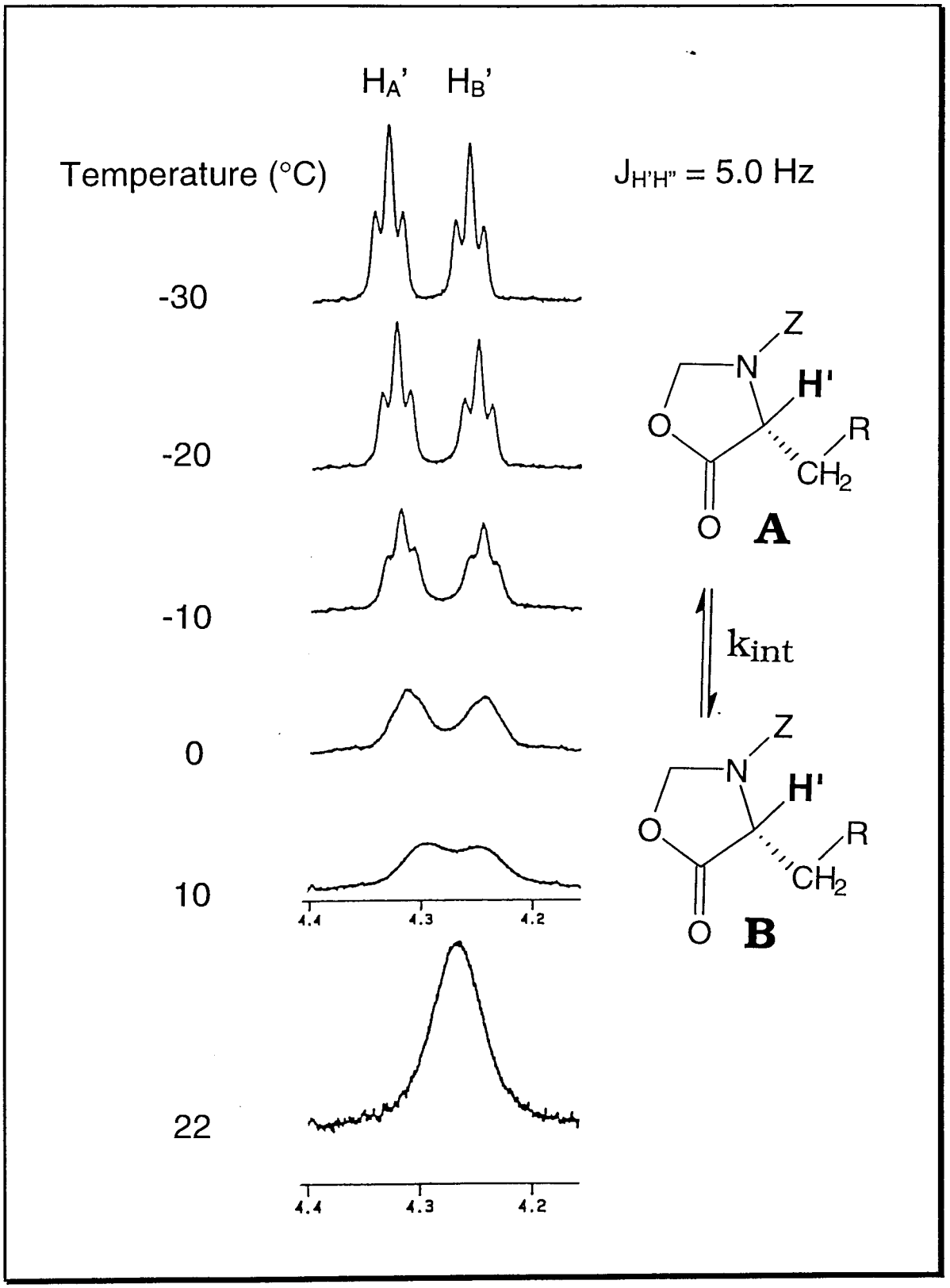


Figure 2.14. Temperature dependence of the ^{1}H NMR spectrum of **9** at 4.4-4.15 ppm

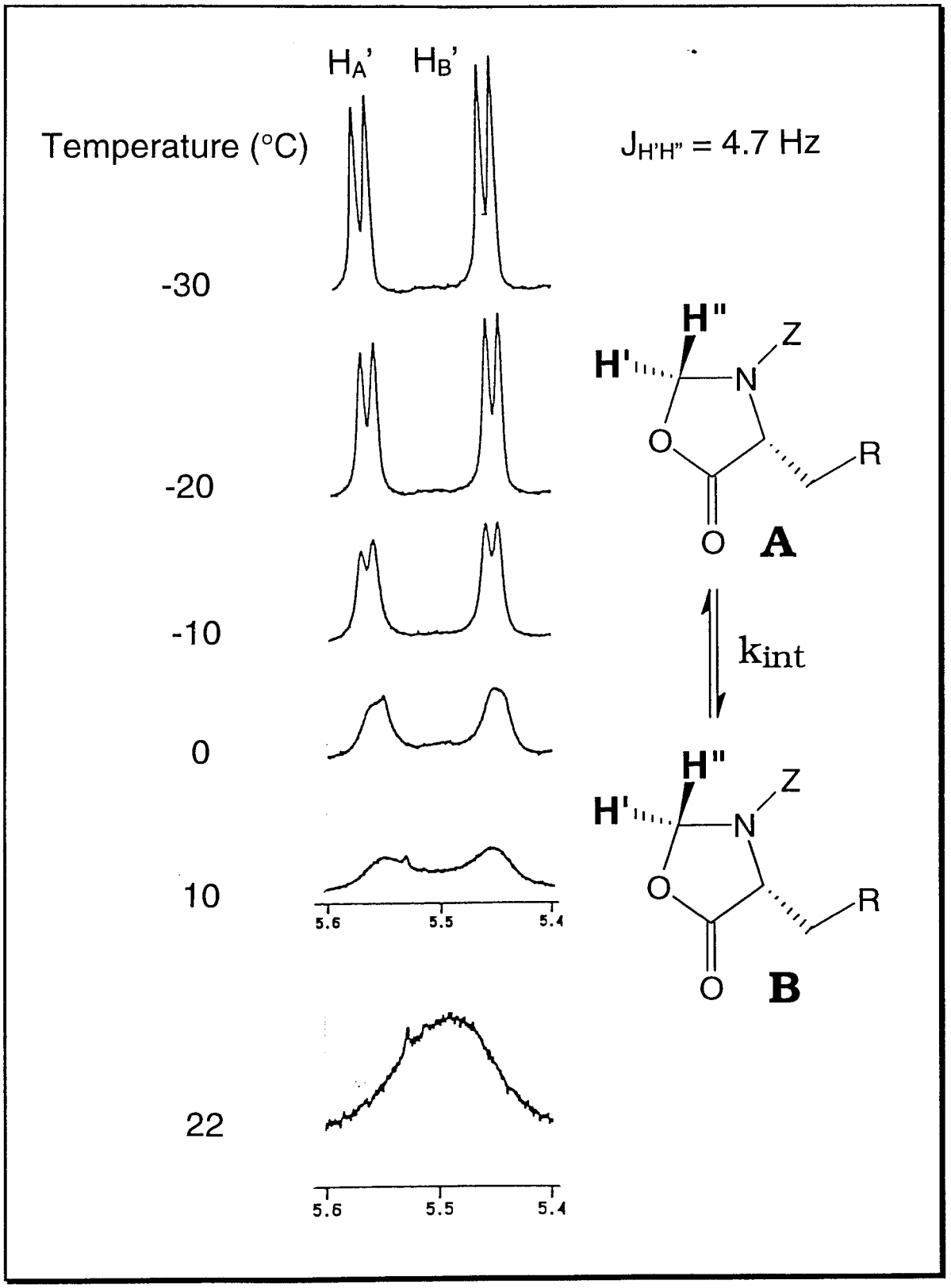


Figure 2.15. Temperature dependence of the ^{1}H NMR spectrum of **9** at 5.6-5.4 ppm

At -30°C the indicated proton in position H_A interconverted or rotated into position H_B in the two different conformers on such a time scale that the NMR experiment could resolve them.

By using equation 3,[49] with a = 1.914 x 10^{-2} to give the free energy in $kJmol^{-1}$, it was possible to estimate an energy barrier of 61 $kJmol^{-1}$ for the process at T_c, noting the estimated coalescence temperature T_c and differences in chemical shifts $\Delta\upsilon_{AB}$ (Table 2.2).

$$\Delta G^{\pm} = aT_c\left[9.972 + \log\left(\frac{T_c}{\Delta\nu_{AB}}\right)\right] \qquad (3)$$

Figure	T_c (K)	$\Delta\nu_{AB}$(Hz)	$\Delta G^{\neq}$($kJmol^{-1}$)
2.14	288 ± 3	10 ± 4	63 ± 2
2.15	288 ± 3	35 ± 2	60 ± 1

Table 2.2. Coalescence temperatures and frequency differences for the spectra of **9**.

The chemical shift differences for each set of resonances were found to be temperature dependent. Graphs were therefore constructed (Figures 2.16 and 2.17) from which the correct frequency $\Delta\nu_{AB}$ at the coalescence temperature T_c was estimated.

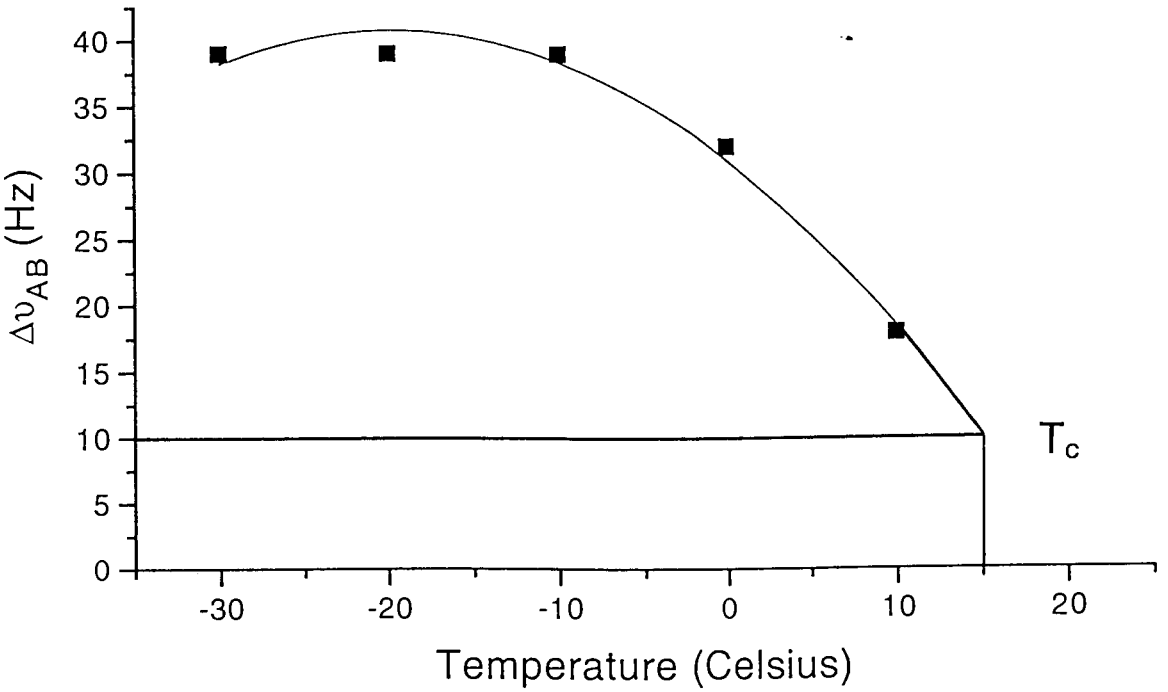


Figure 2.16. Extrapolation of the chemical shift difference $\Delta\nu_{AB}$ (Hz) for one oxazolidinone ^{1}H at the coalescence temperature T_c

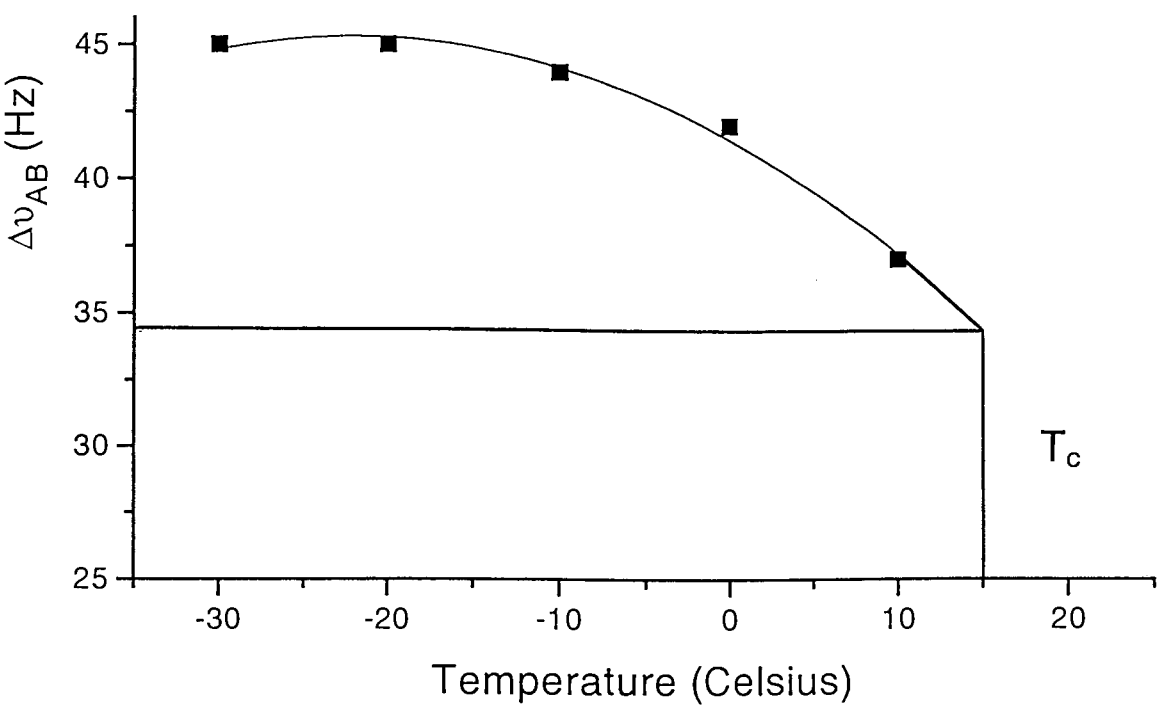
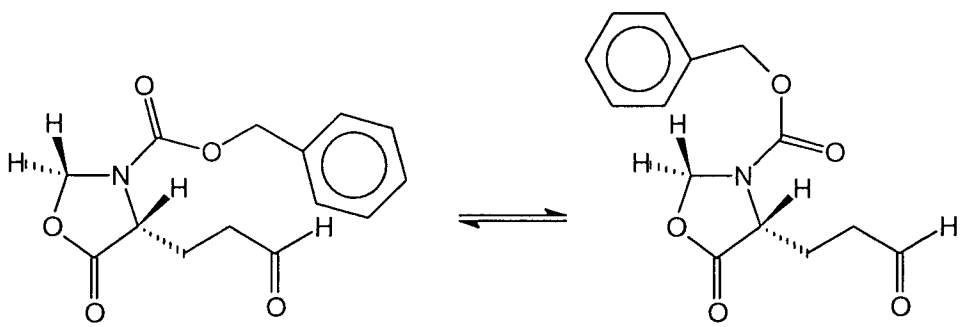
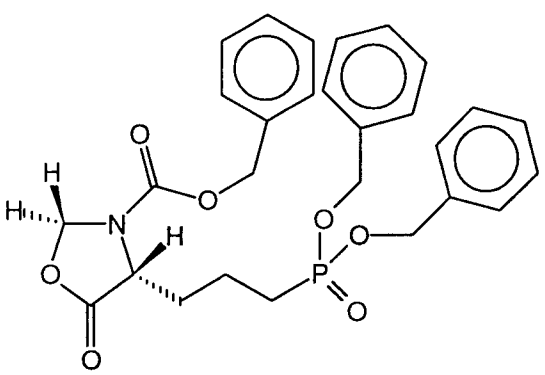


Figure 2.17. Extrapolation of the chemical shift difference $\Delta\nu_{AB}$ (Hz) for two oxazolidinone ^{1}Hs at the coalescence temperature T_c

Considering the structural elements present in **9**, it is possible that the dynamic process is rotation about the nitrogen-carbonyl bond (Figure 2.18). The introduction of the large dibenzyl phosphonate moiety is a reasonable cause of this new restriction.



Easy rotation about C-N bond



Hindered rotation about C-N bond

Figure 2.18. A possible dynamic process leading to broadening of oxazolidinone proton signals at 20°C

2.2.2.2 *Simplification of diastereomer assignments*

2D COSY ^{1}H NMR spectra were required to determine several structures. In particular, the series **19**, **21** and **23** were difficult to assign because each existed as a mixture of diastereomers and exhibited extensive coupling to phosphorous (Figure 2.19).

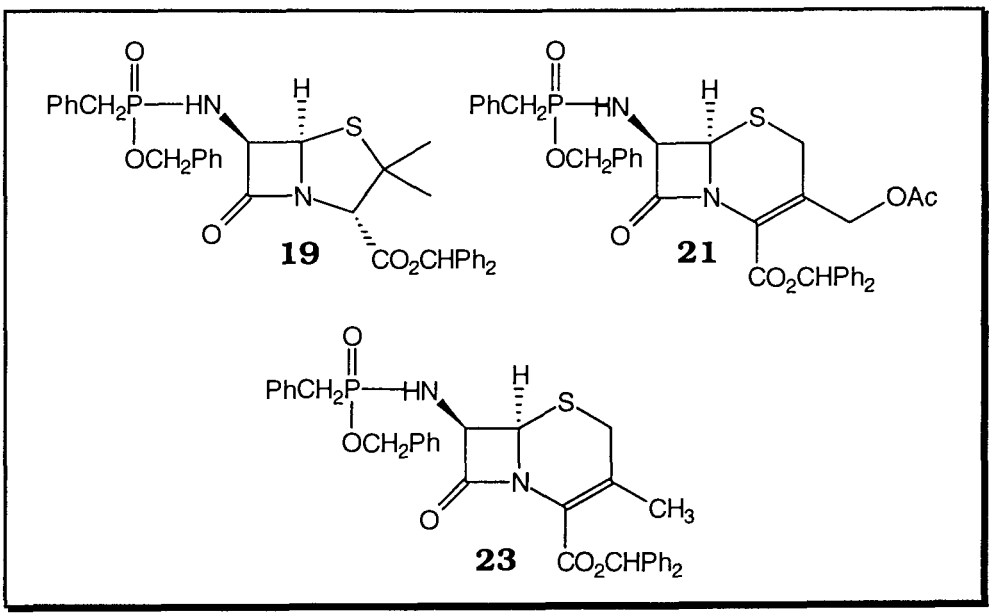


Figure 2.19. Structures of **19**, **21** and **23**

In the case of **19**, assignments were simplified as the two diastereomers shown in Figures 2.21 and 2.22 were separable on silica gel. This entire series had enough structural similarity that, along with ^{31}P decoupling, confident assignments could be made even for the unresolved mixtures of diastereomers **21** and **23**.

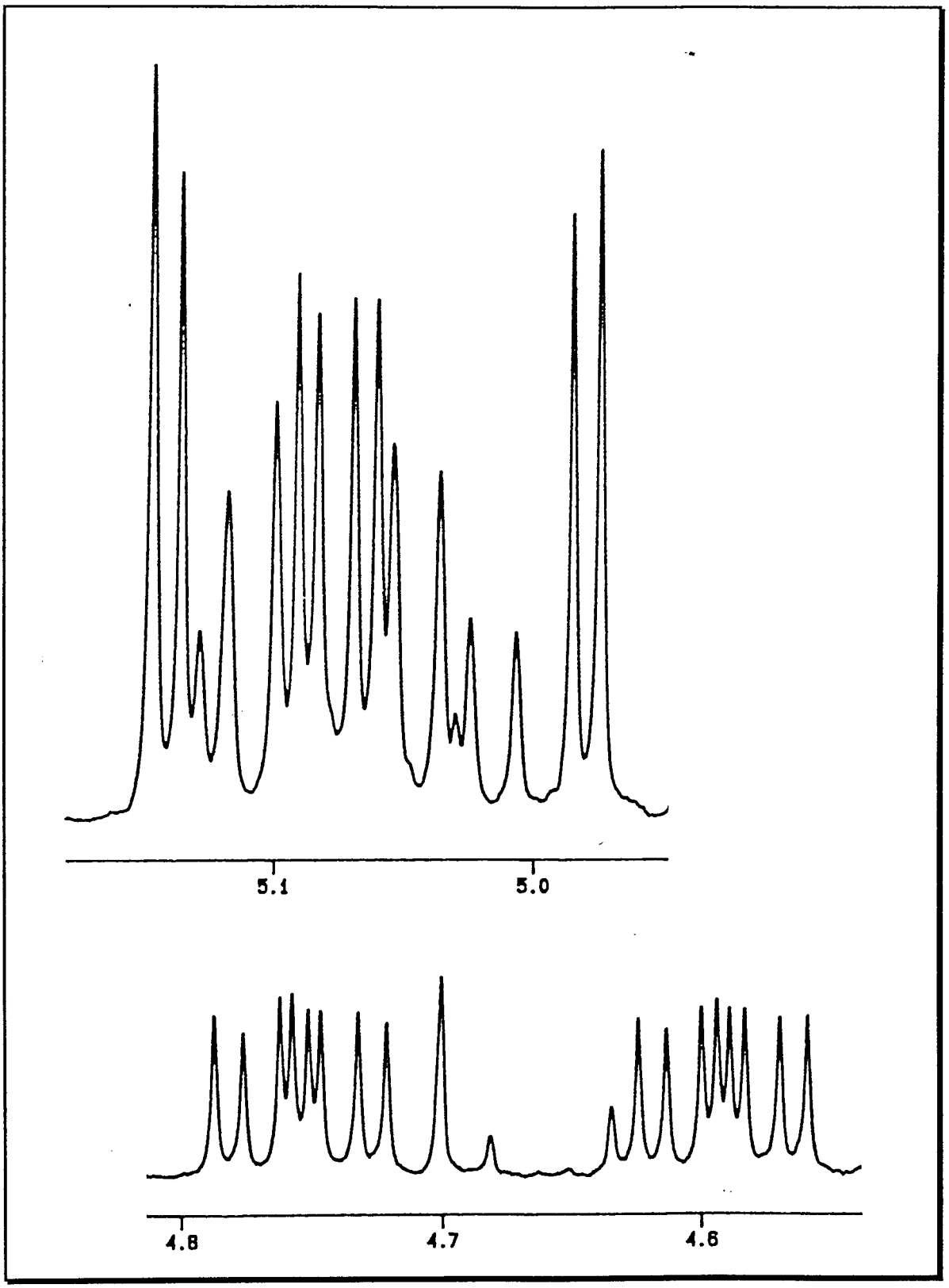


Figure 2.20. Complex splitting in the 400 MHz spectrum of a mixture of diastereomers **19a** and **19b**.

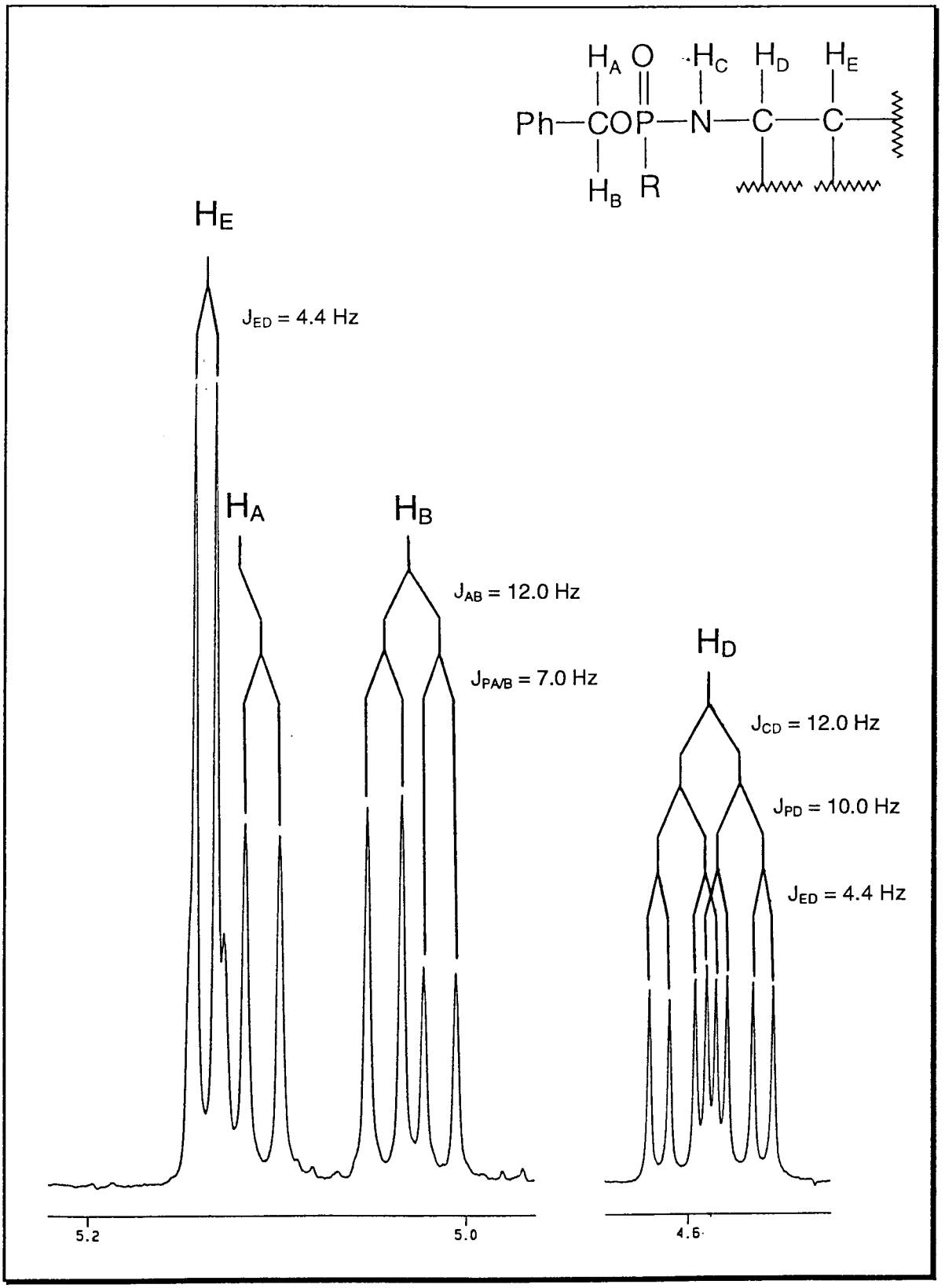


Figure 2.21. Complex splitting and coupling assignments in the 400 MHz spectrum of diastereomer **19a**

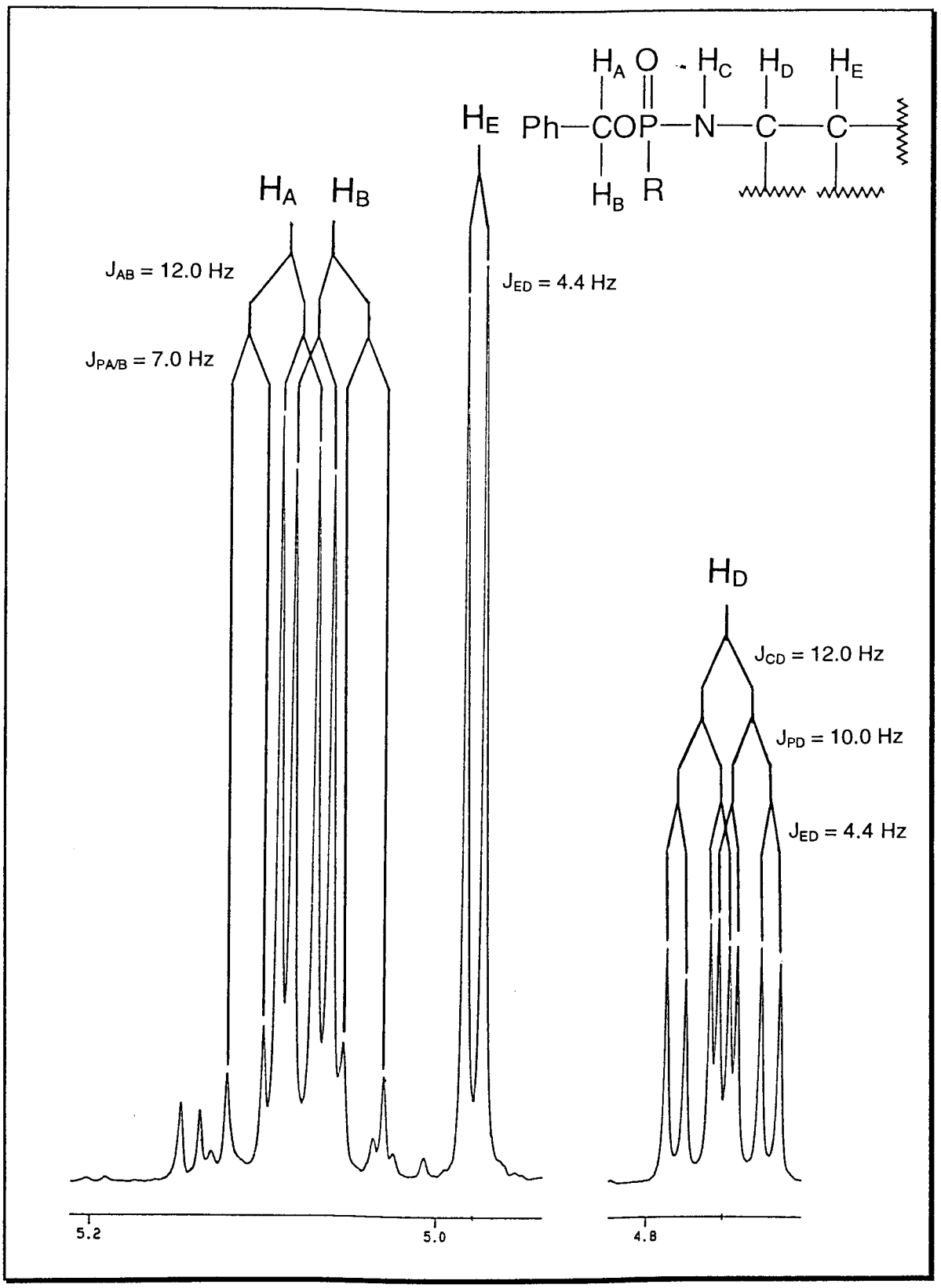


Figure 2.22. Complex splitting and coupling assignments in the 400 MHz spectrum of diastereomer **19b**

Chapter 3

Discussion

3.1 The R-AP5 side chain

The side chain of the hapten **8** is the phosphonamide of R-2-amino-5-phosphonopentanoic acid, R-AP5. The original attempts to synthesize the side chain, via oxazolidinones **29** and **34** (Figure 3.1) resulted in the destruction of the ring and the isolation of one major product.

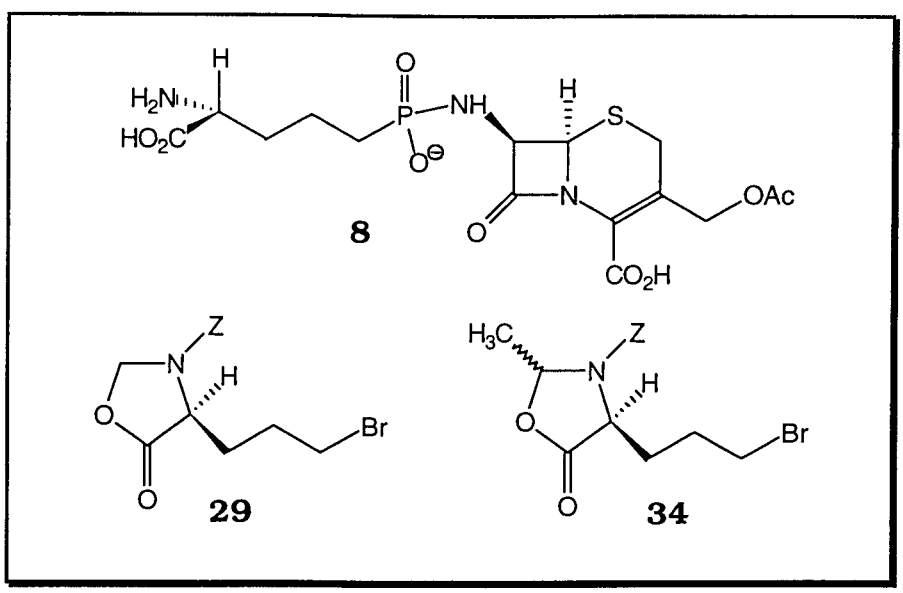


Figure 3.1. Structures of **8**, **29** and **34**

Further analysis indicated that the product of both alkylation reactions is **30** (Figure 3.2). A 2D COSY ^{1}H NMR (Figure 3.3) helped to deduce the structure of **30** by clearly showing the direct coupling of the

benzyloxyamide nitrogen proton at 5.35 ppm, a doublet, and the methine proton of the stereogenic centre at 4.45 ppm, an overlapping doublet of triplets (pseudo quartet).

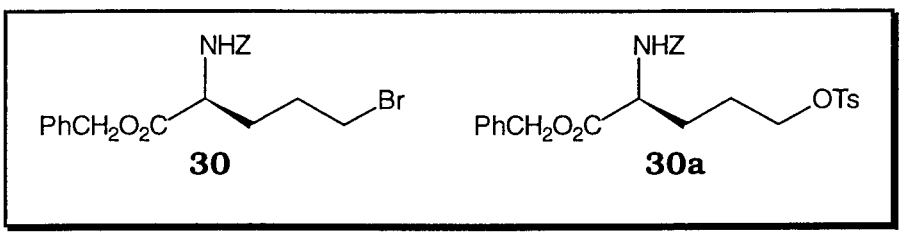


Figure 3.2. Structures of **30** and **30a**.

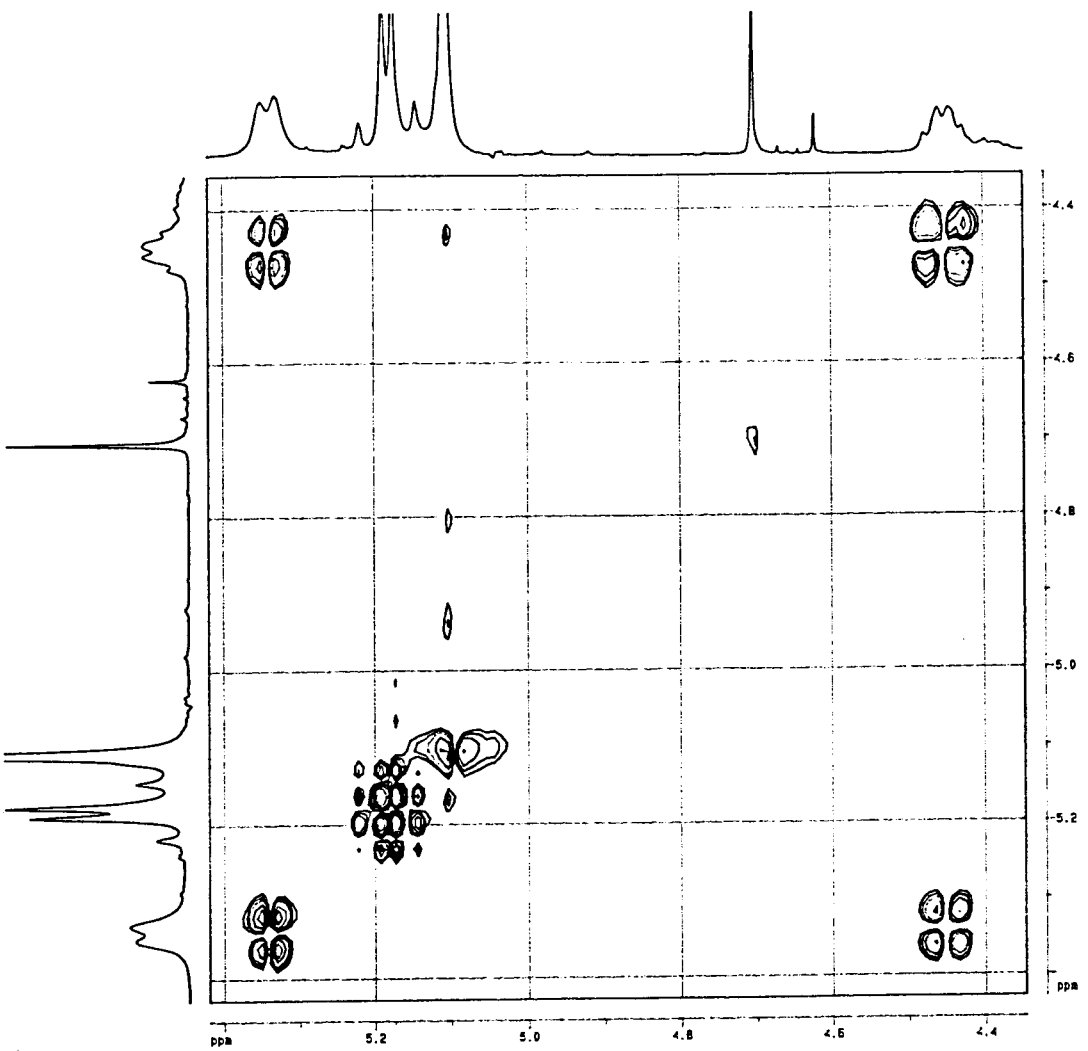


Figure 3.3. 2D COSY ^{1}H NMR of **30**

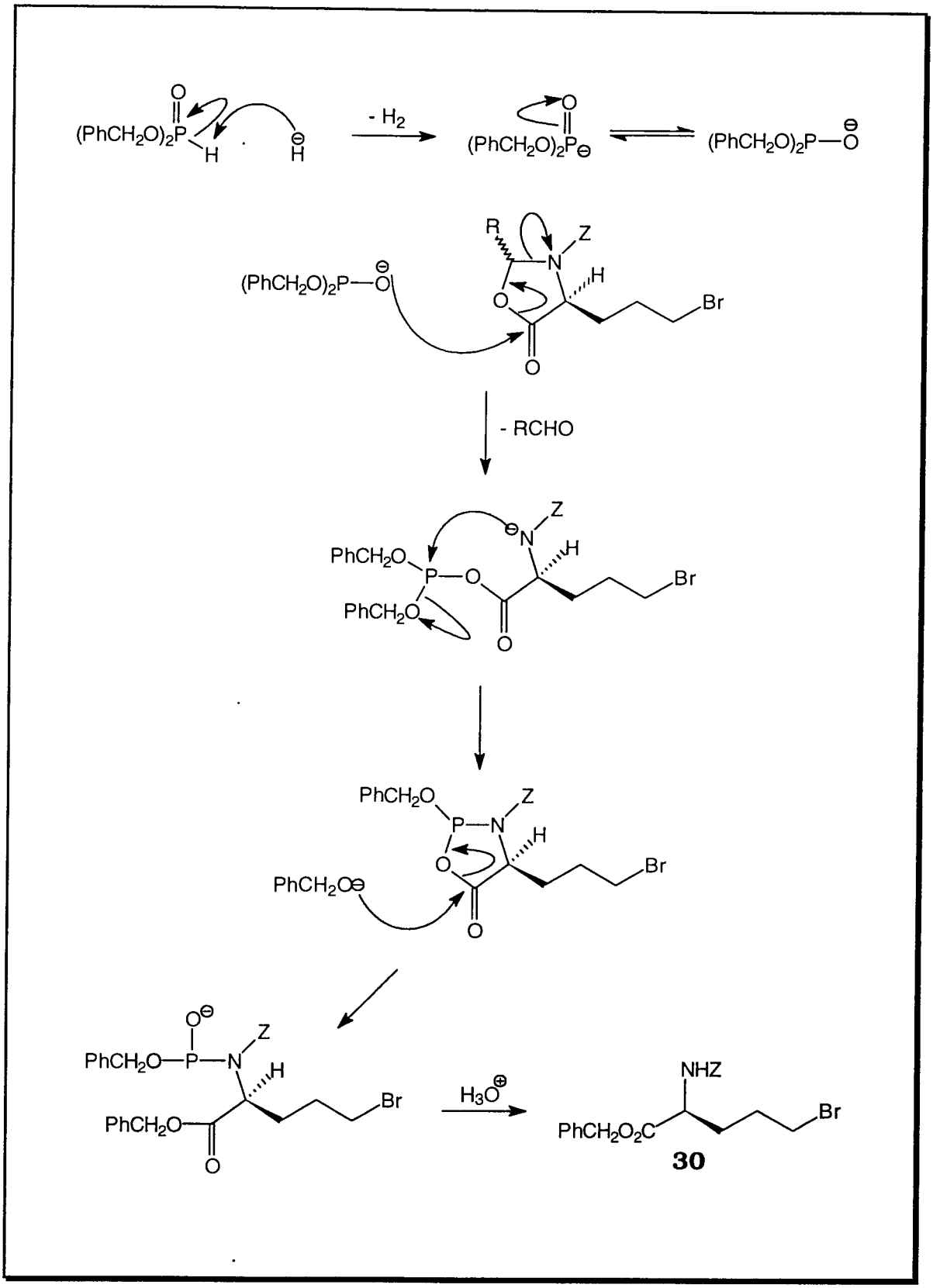
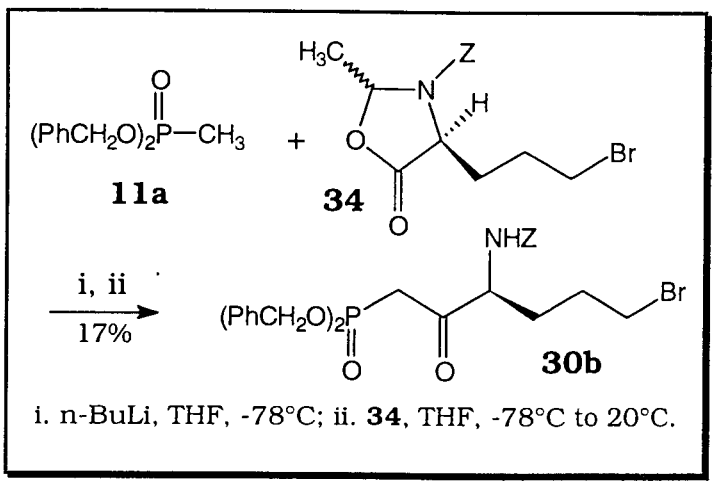


Figure 3.4. Possible mechanism for the formation of **30**

A possible mechanism for the loss of the oxazolidinone ring is outlined in Figure 3.4. This mechanism is supported by the additional finding that the reaction of a homologue of **11** (**11a**) with **34** led to a new compound assigned structure **30b** (Scheme 18).



Scheme 18

The mechanism proposed for this reaction is shown in Figure 3.5.

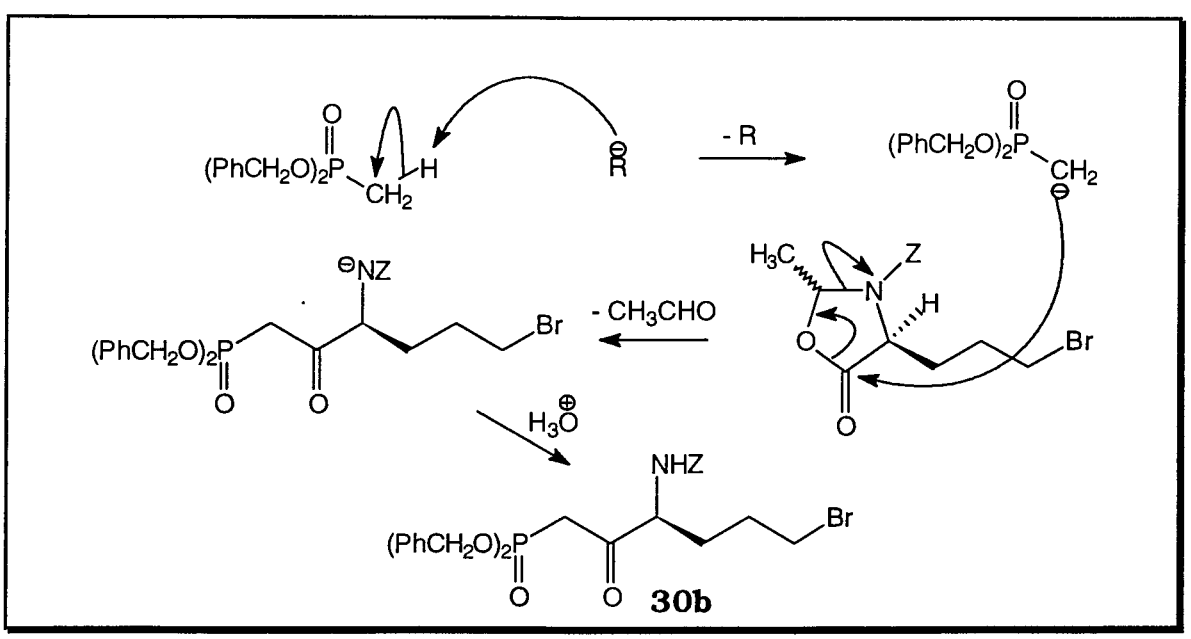


Figure 3.5. Possible mechanism for the formation of **30b**

3.2 α-*Hydroxyphosphonate syntheses*

Alternative methods to the simple S_N2 displacement proposed for **29** and the anion of **11** for phosphorous-carbon bond formation were required (Figure 3.6).

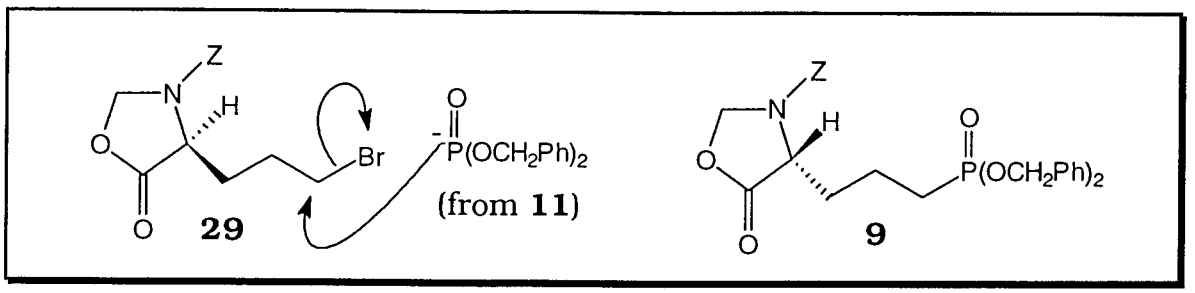
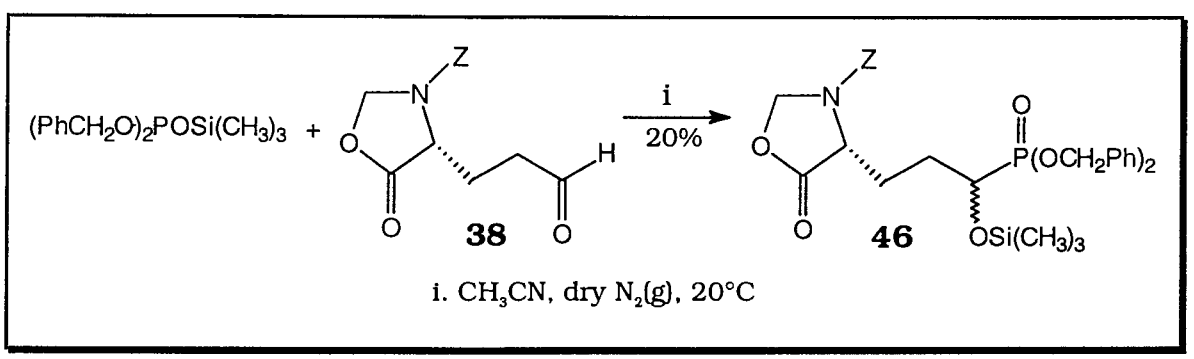


Figure 3.6. Structures of **9** and **29**

The Abramov reaction had found some success in our group[50] and was employed to synthesize the silyloxyphosphonate **46** (Scheme 19), which was ultimately transformed into the desired phosphonate **9**.



Scheme 19

As stated in section 2.1.9, this step was unsatisfactory for several reasons, and an improved procedure using titanium (IV) propoxide[23] was adapted to the precursors **11** and **38**.

This procedure was originally developed to effect enantiofacial additions of phosphites to aldehydes using chiral Lewis acids leading to α-hydroxyphosphonates[23] (Figure 3.7). For our purposes the stereochemistry of addition was irrelevant, because of the eventual removal of the stereogenic centre at the α carbon. Conditions were varied until near quantitative yields were reached using excess phosphonate **11** in toluene.

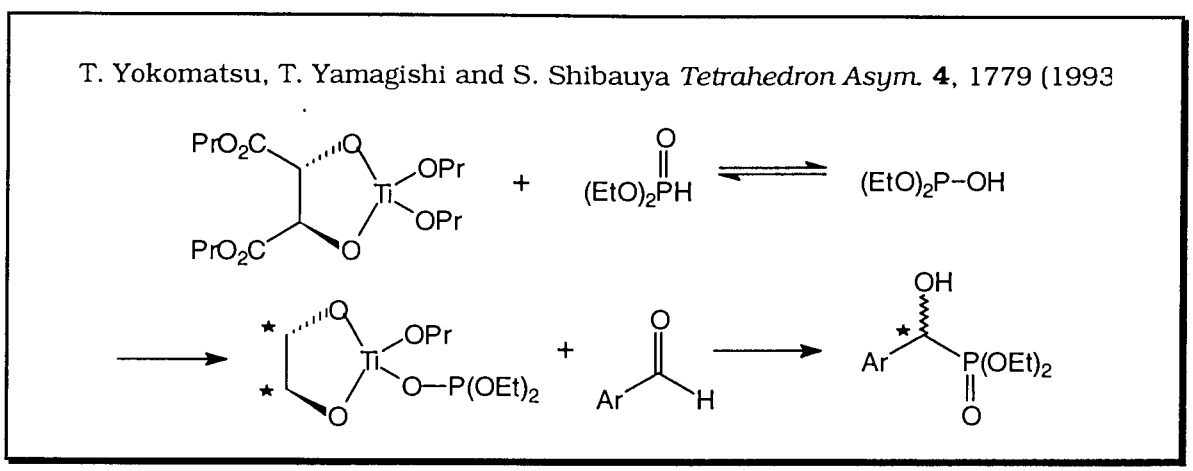


Figure 3.7. Enantioselective addition of diethyl phosphite to aldehydes

3.3 Formation of the phosphonamidates

By far the most successful route to phosphonamidates consists of the reaction of a phosphonochloridate with an amine.[25] The method of Malachowski and Coward[26] recognizes the utility of oxalyl chloride for chlorodeesterification in the presence of complex functionalities.

In the present work these reactions were performed using oxalyl chloride as the solvent to allow monitoring by ^{1}H NMR. This allowed the

benzyl chloride product to be used as a measure of the progress of the reaction. This was especially important in the conversion of **9** to **43**, because of the overlapping signals of the benzyl esters (Figures 3.8, 3.9 and 3.10).

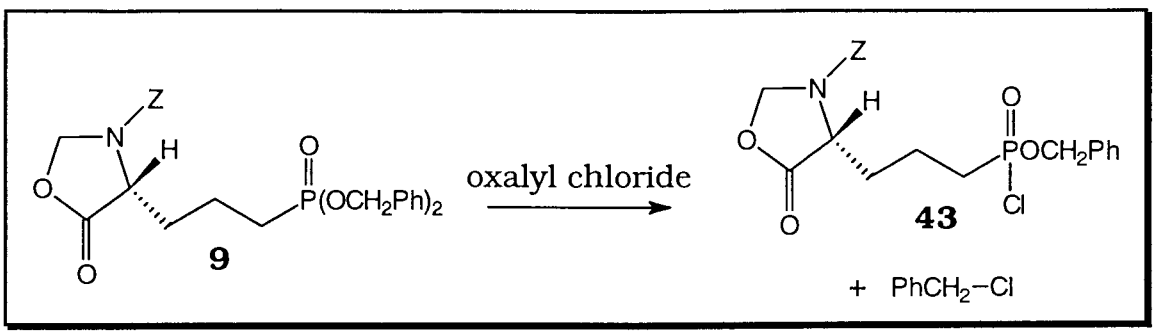


Figure 3.8. Generation of the phosphonochloridate **43**

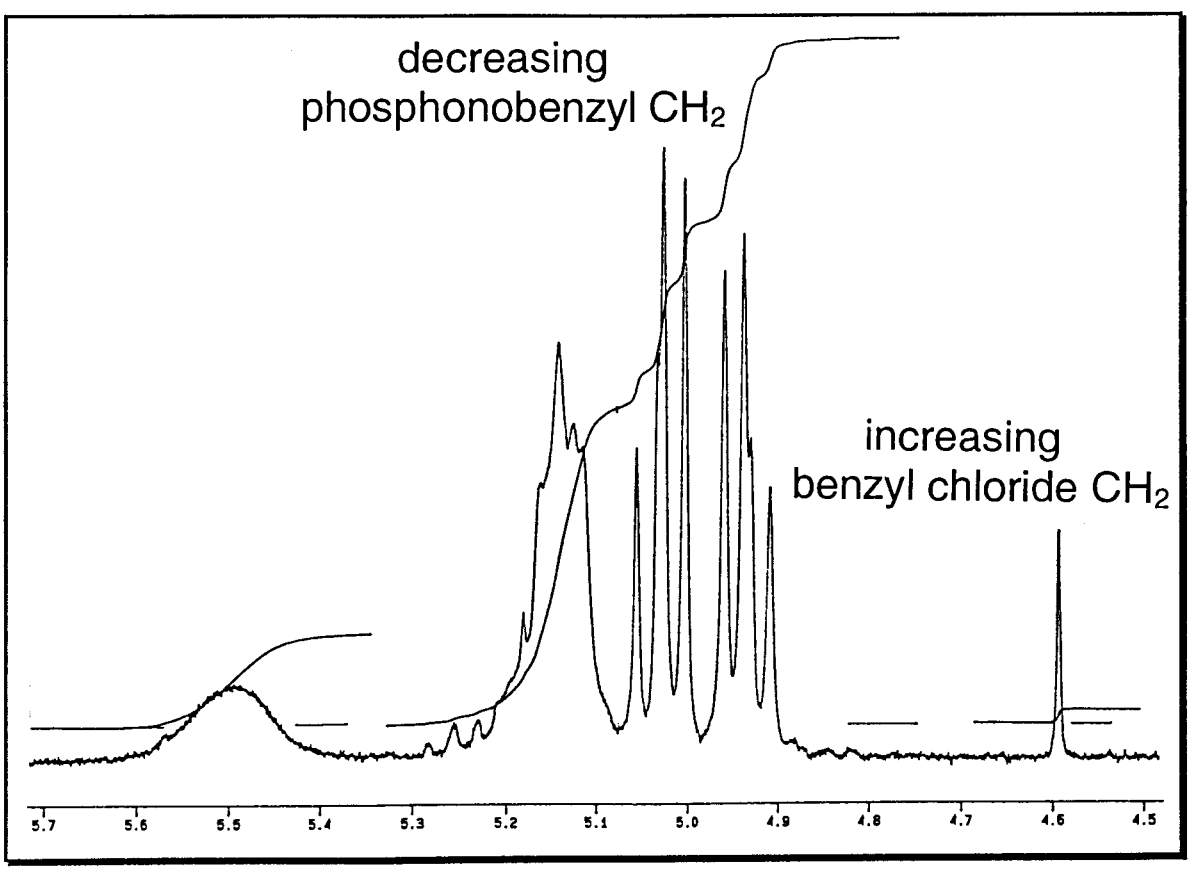


Figure 3.9. ^{1}H NMR spectrum of **9**, **43** and benzyl chloride, 8% conversion

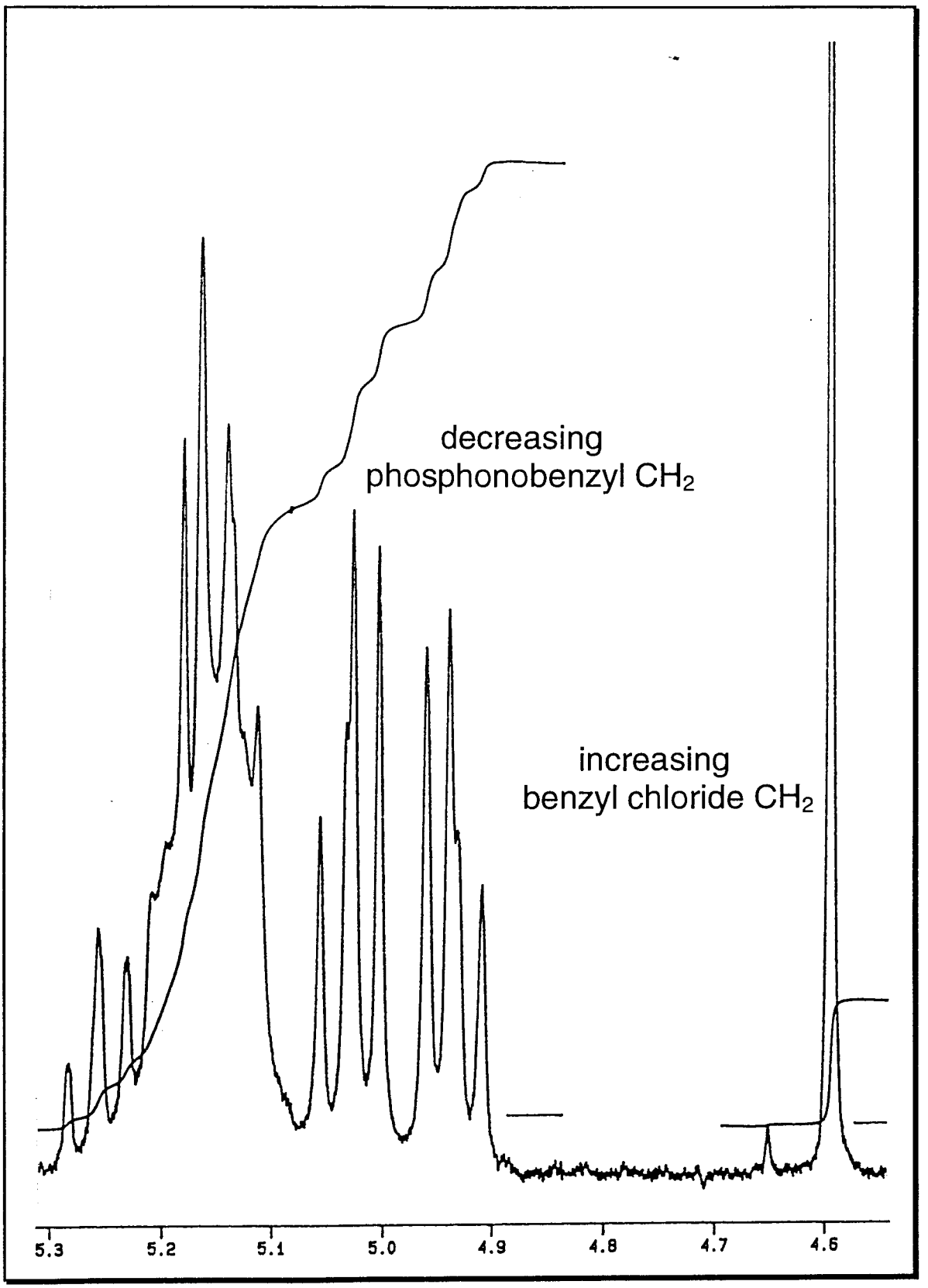


Figure 3.10. ^{1}H NMR spectrum of **9**, **43** and benzyl chloride, 46% conversion

3.4 Deprotection strategies and results

Considering the fragility of the phosphonamide functional group, the literature methods of deprotection involve either strongly basic ester hydrolysis with an alkaline metal hydroxide[45] or neutral hydrogenolysis. With a base-sensitive β-lactam ring attached, hydrogenolysis appeared to be the only practical route for the deprotection of the phosphonamidates **19**, **21** and **23** (Figure 3.11).

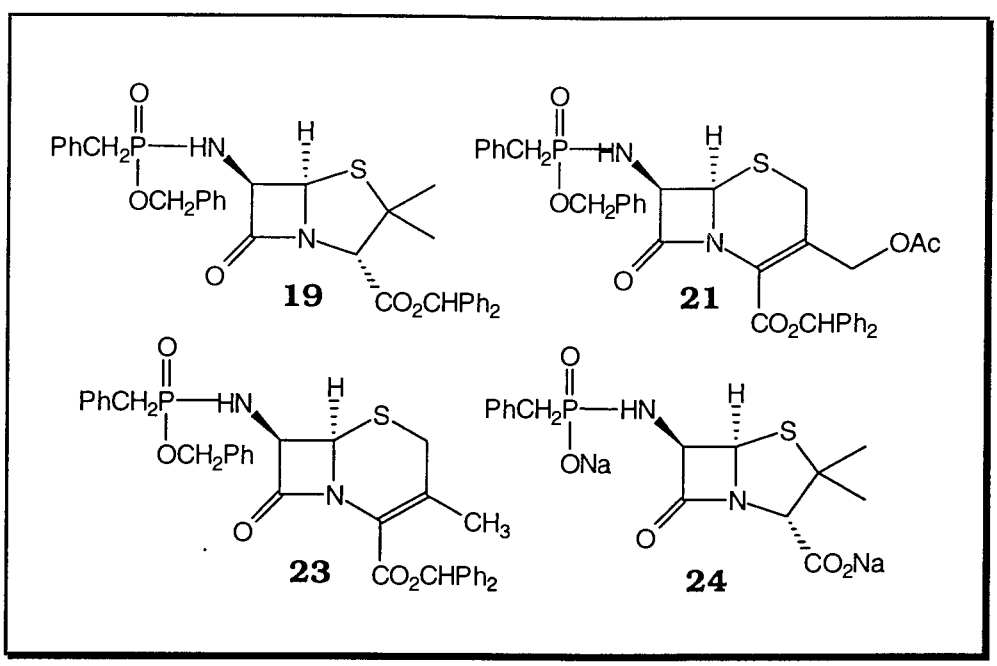


Figure 3.11. Structures of **19**, **21**, **23** and **24**

During the deprotection of **19** to **24** several lyophilizations were required to obtain a clean sample for ^{1}H NMR. By keeping the sample solution close to 0° C a minimum of decomposition occurred and the spectrum of Figure 3.12 was obtained.

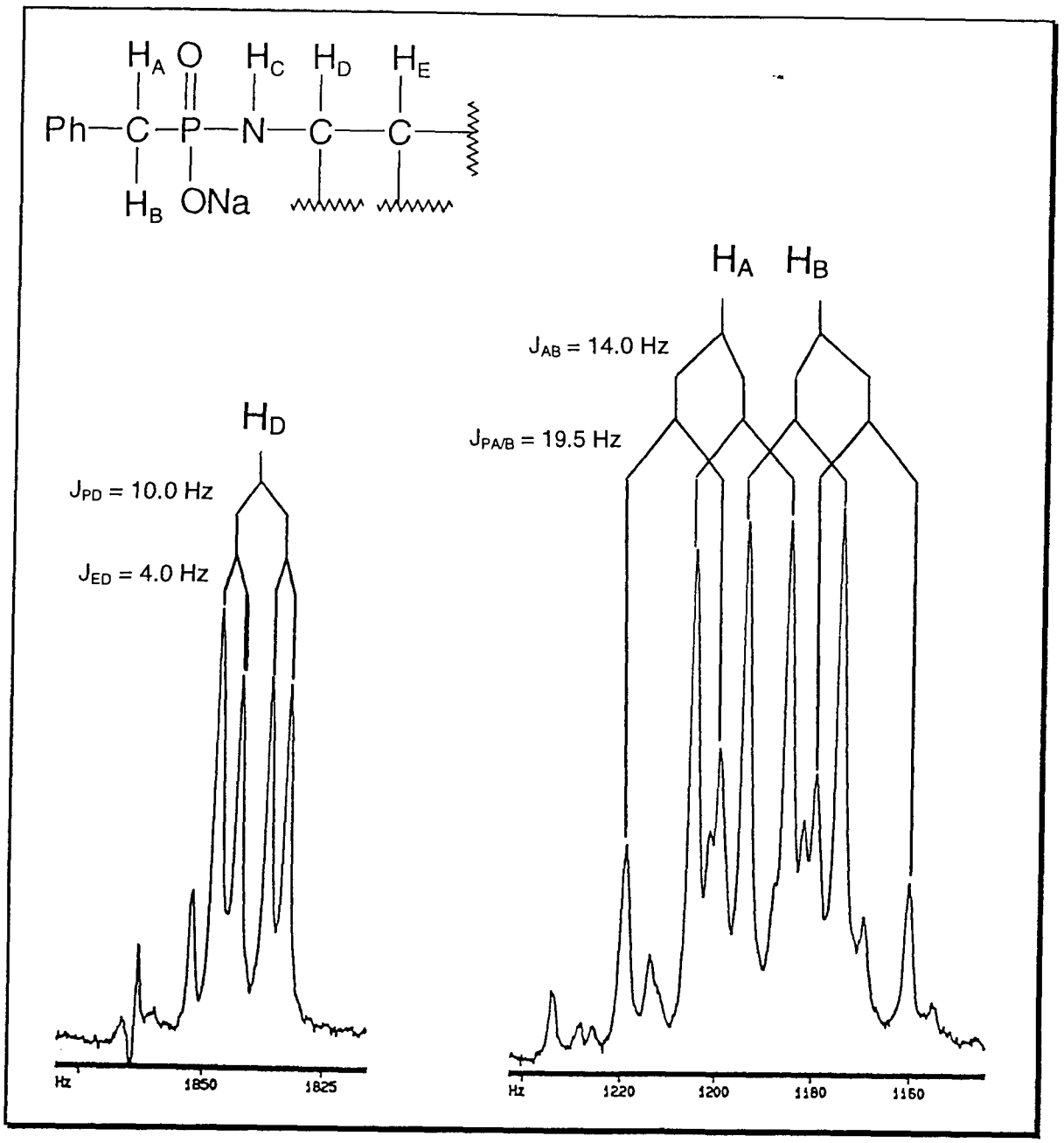


Figure 3.12. ^{1}H NMR spectrum of phosphonamide **24**

The phosphonamide benzyl protons appear as a characteristic AB quartet, with further splitting due to coupling to ^{31}P. The β-lactam proton vicinal to the phosphonamide nitrogen appears as a doublet of doublets, due to coupling to phosphorous and the other β-lactam proton.

3.5 Kinetics of hydrolysis

It is believed, both from published reports and our own study, that the hydrolysis of **17** depicted in Figure 2.6 can be represented as follows;

$$\mathbf{A} + D_3O^{\oplus} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathbf{B} + D_2O \xrightarrow{k_2} \mathbf{C} + \mathbf{D}$$

The ^1H NMR spectra involved in the rate constant calculations show **A** and **B** as a single peak, due to their rapid equilibration. The linearity of the logarithmic plots indicates that the hydrolysis is first-order in **B** and allows the estimation of 88 $kJmol^{-1}$ as the activation energy for hydrolysis in D_2O.

For comparison, the activation energy for the hydrolysis of the aryl phosphonamide **E**[27] in acetate buffer (Figure 3.13) was found to be 50.1 $kJmol^{-1}$. It is difficult to compare these reactions in a meaningful way, as there are many differences in the conditions employed in each case, including pH, solvent media and substrate structure. Additionally, the solvent isotope effect for this class of reaction is quite large;[27] all of our experimental data were obtained in D_2O.

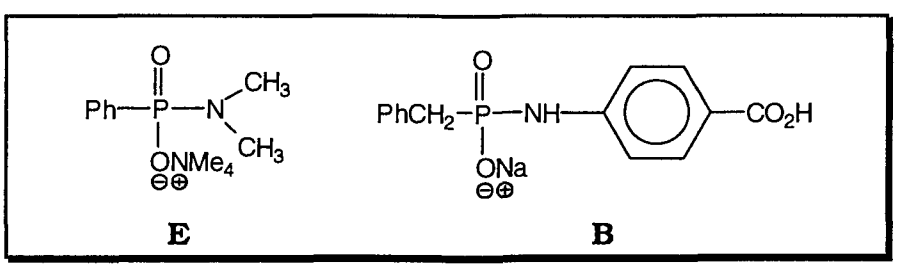


Figure 3.13. Comparison of phosphonamide structure

Chapter 4

Conclusions

The syntheses of phosphonamidates ranging from the simple **16** to the complex **19**, **21** and **23** has enabled a greater understanding of the conditions and reagents required for successful phosphorous-carbon and phosphorous-nitrogen bond formation. The deprotection of **16** to **17** and **19** to **24** has proven the utility of the hydrogenolysis strategy and illustrated the lability of the phosphonamides in aqueous acid.

Future work on this project will require a more thorough investigation of the coupling reaction between **20** and **43**. It will also be of interest to deprotect and purify the phosphorous analogues of penicillin G, cephalosporin G and desacetoxycephalosporin G and evaluate these compounds as antibacterial agents.

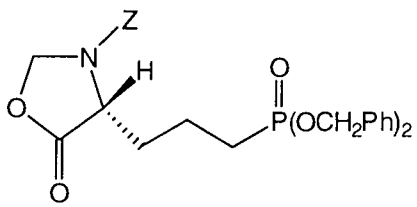
Chapter 5

Experimental

Solvents were dried by standard procedures and distilled prior to use. ^{1}H, ^{13}C and ^{31}P NMR were obtained on either a Bruker model SY-100, AMX 400 or 600 spectrometer. Chemical shifts are recorded in ppm downfield from trimethylsilane for both proton and carbon spectra, and phosphoric acid for phosphorous spectra. Melting points were determined on a Fischer-Johns melting point apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer 599B spectrophotometer (neat film, 1% KBr pellet or 1% solution). Mass spectra were obtained on a Hewlett-Packard 5985 GC/MS/IS system, and operated at 70eV in electron impact (EI) or chemical ionization (CI) mode. Microanalyses were carried out on a Carlos Erba model 1106 elemental analyzer. Optical rotations were determined using a Rudolph Automatic polarimeter model Autopol II, with a cell length of 10cm. Concentrations are reported in g/100mL solvent. Analytical thin layer chromatography was performed on precoated Merck silica gel 60 F-24 plates with aluminium backing. Spots were observed under ultraviolet light and were visualized with 1% ceric sulfate or ninhydrin solution. Column chromatography was carried out using 230-400 mesh silica gel (Merck). Compound **41** was made available by Dr. Blair Johnston.

Note that in many cases FAB and CI mass spectroscopy failed to give molecular ions or interpretable mass spectra. A variety of compounds apppeared to decompose rapidly when analyzed.

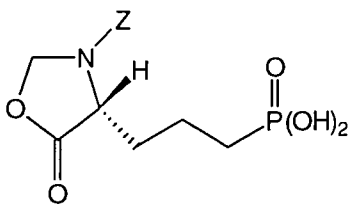
Preparation of 9



Tributyltin hydride (300 μL, 294 mg, 1.0 mmole) was injected into a solution of **40** (520 mg, 0.8 mmoles) in dry benzene (8 mL). The solution was refluxed for 2 h, and then cooled and evaporated to give a golden oil. Column chromatography (75% ethyl acetate-hexane) afforded the product **9** (272 mg, 65%) as an oil. **^{1}H NMR** ($CDCl_3$) δ (243K): 7.30 (15 H, m, aromatic), 5.57 (1 H, d, oxazolidinone C<u>H</u>H, 4.7 Hz (one conformer)), 5.46 (1 H, d, oxazolidinone C<u>H</u>H, 4.7 Hz (one conformer)), 5.05 (7 H, m, benzyloxy CH_2, two phosphonobenzyloxy CH_2, oxazolidinone CH<u>H</u>), 4.34 (1 H, t, α CH, 5.1 Hz (one conformer)), 4.27 (1 H, t, α CH, 5.1 Hz (one conformer)), 1.80 (6 H, m, alkyl $(CH_2)_3$). **^{13}C NMR** ($CDCl_3$) δ: 171.84 (oxazolidone carbonyl), 152.90 (urethane carbonyl), 136.45, 136.39, 135.42, 128.75, 128.64, 128.46, 128.34, 128.00 (aromatic C, CH), 77.89 (oxazolidinone CH_2), 68.04 (benzyloxy CH_2), 67.25 (d, J_{PC}=6.3 Hz, phosphonobenzyl CH_2), 54.56 (α CH), 31.40 (alkyl P CH_2 $CH_2\underline{C}H_2$), 25.71(d, J_{PC}=141.6 Hz, alkyl PCH_2), 17.77 (d, J_{PC}=4.5 Hz, alkyl P $CH_2\underline{C}H_2$). **^{31}P NMR {^{1}H}** δ: 32.41. **IR** (film): 1801 (s), 1716 (s), 1240 (s) cm^{-1}. **Calcd. for $C_{28}H_{30}NO_7P$•0.5 H_2O**: C, 63.24; H, 5.86; N, 2.63.

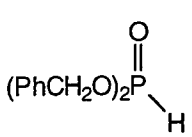
Found: C, 63.56; H, 6.00; N, 3.07. **Mass spectrum**: 523 (M+), 495, 432, 326. $[\alpha]_D^{20}$ (c 1.9, $CHCl_3$): -46.

Preparation of 10



Trimethylchlorosilane (100 μL, 85.6 mg, 0.79 mmoles) was slowly added by syringe to a solution of **9** (195 mg, 0.37 mmoles) and dry sodium iodide (117 mg, 0.78 mmoles) in dry acetonitrile (1.2 mL). After 1 h of stirring the mixture was filtered and the filtrate was evaporated to a yellow oil (300 mg). Hexane (10 mL) and water (5 mL) were added and the layers were separated. Additional acetonitrile (2 mL) and additional water (5 mL) were added to the aqueous layer. This was washed with hexane (10 mL) and the resulting emulsion was centrifuged. Acetonitrile (10 mL) was added to the aqueous layer and the solution was evaporated to give **10** as a yellow oil (120 mg, 94%). **^{1}H NMR** ($CDCl_3$) δ: 9.63 (2 H, s, acid $P(OH)_2$), 7.30 (5 H, s, aromatic), 5.47 (1 H, d, oxazolidinone C<u>H</u>H, 3.50 Hz), 5.18 (3 H, m, benzyloxy CH_2, oxazolidinone CH<u>H</u>), 4.32 (1 H, m, α CH), 1.70 (6 H, m, alkyl $(CH_2)_3$). **IR** (film) υ: 2924 (s), 1800 (s), 1714 (s), 1135 (s), 1027 (s) cm^{-1}.

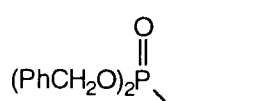
Preparation of 11



Benzyl alcohol (35.0 mL, 36.8 g, 340 mmoles) was slowly injected into an ice-cold solution of phosphorous trichloride (10.0 mL, 15.7 g, 114

mmol) and dry pyridine (26.0 mL, 27.3 g, 345 mmoles) in dry methylene chloride (350 mL). A heavy white precipitate formed after 10 mL of alcohol had been added. After 15 min, the solution was allowed to warm to room temperature, stirred for 20 h, and then filtered. The filtrate was concentrated to 80 mL under reduced pressure and filtered again. The new filtrate was diluted to 150 mL with methylene chloride and washed successively with 1 M hydrochloric acid (2 x 100 mL), saturated sodium bicarbonate (2 x 100 mL), saturated sodium chloride (100 mL), dried over anhydrous magnesium sulfate and evaporated to a yellow oil (40 g). Column chromatography (45% ethyl acetate-hexane) of a 15 g portion of the oil gave **11** (14.4 g, 96% yield). **^{1}H NMR** ($CDCl_3$) δ: 7.37 (10 H, m, aromatic), 6.95 (1 H, d, PH, 704 Hz) ,5.07 (4 H, d, benzyloxy CH_2, 9.3 Hz). **^{13}C NMR** ($CDCl_3$) δ: 128.73, 128.69, 128.04 (aromatic C, CH), 67.33 (d, J_{PC}=5.0 Hz, benzyl C). **IR** (film) υ: 1259 (s), 962 (s) cm^{-1}. **Calcd for $C_{14}H_{15}O_3P$**: C, 64.12; H, 5.77. **Found**: C, 63.79; H, 5.92.

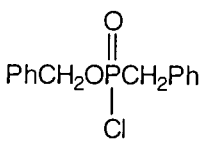
Preparation of 12



A solution of **11** (3.32 g, 12.6 mmoles) in dry tetrahydrofuran (30 mL) was added during 30 min to a suspension of sodium hydride (560 mg, 14.0 mmoles) in dry tetrahydrofuran (30 mL). Stirring was continued for 3 h under nitrogen, and benzyl bromide (1.51 mL, 2.17 g, 12.6 mmoles) in dry tetrahydrofuran (2.0 mL) was then added quickly. Stirring was continued for 20 h at room temperature and the reaction was quenched by pouring into 1:1 methylene chloride-water (100 mL). The layers were separated, the aqueous portion was extracted with methylene chloride (100 mL), and the combined organic extaracts were washed with saturated sodium chloride, dried over anhydrous magnesium sulfate and evaporated to a yellow oil. Column chromatography (45% ethyl acetate-

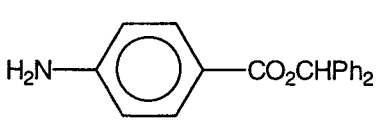
hexane) afforded **12** as a pale yellow oil (3.21 g, 72%). **^{1}H NMR** ($CDCl_3$) δ: 7.29 (15 H, m, aromatic), 4.91 (4 H, d, benzyloxy CH_2, 8.0 Hz), 3.19 (2 H, d, benzyl CH_2, 21.6 Hz). **^{13}C NMR** ($CDCl_3$) δ: 136.47, 136.41, 131.35, 131.26, 128.92, 129.86, 128.57, 128.54, 128.50, 128.30, 127.89, 126.95, 126.91 (aromatic C, CH), 67.64 (d, J_{PC}=6.7 Hz, benzyloxy CH_2) 34.66 (d, J_{PC}=37.2 Hz, benzyl CH_2). **IR** (film) υ : 1251 (s), 996 (s) cm^{-1}. **Calcd for $C_{21}H_{21}O_3P$**: C, 71.58; H, 6.01 **Found**: C, 70.99; H, 6.07.

Preparation of 13



Oxalyl chloride (457 μL, 667 mg, 5.3 mmoles) was injected with rapid stirring under nitrogen at 40° C into a flask containing **12** (1.09 g, 3.1 mmoles). A vigorous reaction occurred with gas evolution, and the mixture turned a brilliant yellow. After 3 h an aliquot (~10 μL) was diluted to 1 mL with deuterated chloroform and its ^{1}H NMR spectrum indicated the reaction was 70% complete. The remainder of the reaction mixture was then evacuated at 60° C/10 torr and the resulting yellow oil was used immediately to prepare **17**, **19**, **21** or **23**. **^{1}H NMR** ($CDCl_3$) δ: 7.29 (10 H, m, aromatic), 5.19 (2 H, dd, benzyloxy CH_2, 8.5, 3.4 Hz), 3.57 (2 H, d, benzyl CH_2, 20 Hz).

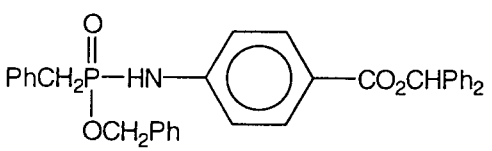
Preparation of 15



Diphenyldiazomethane (1.16 g, 5.97 mmoles) was added at room temperature under nitrogen to a stirred solution of p-aminobenzoic acid (492 mg, 4.00 mmoles) in methylene chloride (16 mL) and methanol (8 mL). After 20 h of stirring the purple color had faded to yellow. The solvent was removed under reduced pressure and the residue was

dissolved in methylene chloride (10 mL). This solution was washed with 1 M hydrochloric acid (2 x 10 mL) and saturated sodium bicarbonate (10 mL), dried over anhydrous magnesium sulfate and evaporated to leave a solid (1.44 g). Crystallization from ether-hexane afforded **15** (800 mg, 66%), m.p. 138-140°C. **^{1}H NMR** ($CDCl_3$) δ: 8.00 (2 H, d, aromatic, 8.8 Hz), 7.38 (10 H, m, aromatic), 7.08 (1 H, s, benzhydryl CH), 6.75 (2 H, d, aromatic, 8.8 Hz), 4.20 (2 H, s, amine NH_2). **^{13}C NMR** ($CDCl_3$) δ: 165.55 (ester carbonyl), 140.75, 131.81, 128.42, 127.71, 127.10 (aromatic C, CH), 113.76 (benzhydryl CH). **IR** (KBr) υ: 3364 (m), 1692 (s), 1267 (s) cm^{-1}.

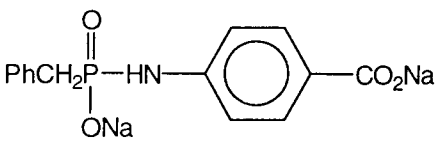
Preparation of 16



Under nitrogen at room temperature, a solution of **13** (505 mg, 1.8 mmoles) in methylene chloride (500 μL) was added via syringe to a solution of **15** (600 mg, 2.01 mmoles) and dry, freshly distilled triethylamine (260 μL, 189 mg, 1.9 mmoles) in dry methylene chloride (3 mL). The solution was stirred for 24 h and then washed with water (15 mL), saturated sodium bicarbonate (15 mL), dried over anhydrous magnesium sulfate and evaporated to a golden oil (1.84 g). Column chromatography (60% ethyl acetate-hexane) gave **16** (250 mg, 27% based on **13**) as a pale yellow solid. **^{1}H NMR** ($CDCl_3$) δ: 8.02 (2 H, d, aromatic, 8.8 Hz), 7.30 (20 H, m, aromatic), 7.10 (1 H, s, benzhydryl CH), 6.98 (2 H, d, aromatic, 8.8 Hz), 5.90 (1 H, s, NH), 5.03 (2 H, m, benzyloxy CH_2), 3.32 (2 H, d, benzyl CH_2, 20.7 Hz). **^{13}C NMR** ($CDCl_3$) δ: 165.20 (ester carbonyl), 145.05, 140.53, 135.76, 131.63, 130.99, 129.94, 129.88, 128.62, 128.54, 128.16, 127.91, 127.20, 127.18, 123.38 (aromatic C, CH), 116.81 (d, J=6.0 Hz, benzhydryl CH), 64.44 (d, J_{PC}=6.8 Hz,

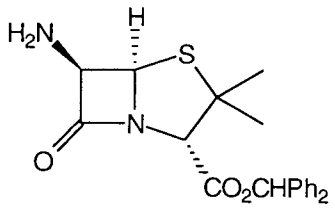
benzyloxy CH_2), 34.31 (d, J_{PC}=24.3 Hz, benzyl CH_2). **IR** (film) υ: 3032 (m), 1713 (s), 1267 (s), 1214 (s), 952 (s) cm^{-1}. **Calcd for $C_{34}H_{30}NO_4P$**: C, 74.58; H, 5.52; N, 2.56 **Found**: C, 74.27; H, 5.55; N, 2.40.

Preparation of 17



A two-necked vessel containing a mixture of **16** (106 mg, 0.19 mmole), sodium bicarbonate (36 mg, 0.43 mmole) and 10% palladium on carbon (120 mg) in tetrahydrofuran (20 mL) and water (6 mL) was fitted with a rubber septum and balloon. The system was purged thrice with nitrogen and thrice with hydrogen and was then pressurized with hydrogen to inflate the balloon. The mixture was stirred rapidly for 1 h and, after the release of the pressure, filtered through a 3 cm pad of washed Celite. The black solids were rinsed with tetrahydrofuran (10mL), water (10 mL) and tetrahydrofuran (10 mL), and the combined liquids were concentrated to remove most of the tetrahydrofuran and then lyophilized. The fluffy grey residue was dissolved in ice-cold water (20 mL) and shaken with ethyl acetate (20 mL). The emulsion was centrifuged and the aqueous layer filtered through a tissue plug and lyophilized to give **17** as a fluffy yellowish powder (83 mg). **^{1}H NMR** (D_2O) δ: 7.70 (2 H, d, aromatic, 8.0 Hz), 7.17 (5 H, m, aromatic), 6.98 (2 H, d, aromatic, 8.0 Hz), 3.13 (2 H, d, benzyl CH_2, 19.2 Hz). **IR** (KBr) υ: 3384 (s), 1607 (s), 1141 (s) cm^{-1}.

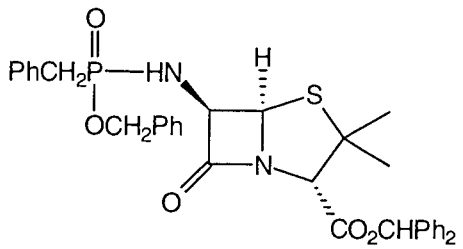
Preparation of 18



A solution of diphenyldiazomethane (1.0 g, 5.1 mmoles) in dry methylene chloride (10 mL) was added to a stirred suspension of 6-aminopenicillanic acid (1.06 g, 5.0 mmoles) in dry methylene chloride (25 mL) and acetonitrile (35 mL), and the mixture was refluxed for 24 h. The purple mixture turned yellow and the solid dissolved. The solvent was evaporated under reduced pressure and the resulting yellow oil was dissolved in methylene chloride (10 mL). p-Toluenesulfonic acid monohydrate (0.96 g, 5.6 mmoles) in dry acetone (10 mL) was added in one portion, to form a crystalline precipitate. Methylene chloride (20 mL) was added and the mixture was transferred to a beaker containing diethyl ether (100 mL). The crystals were collected by filtration, washed with cold methylene chloride and dried to give the p-toluenesulfonic acid salt of **18** (1.90 g, 69%), m.p.158-162° C (decomposes). The base was liberated by shaking the salt with methylene chloride and saturated sodium bicarbonate and evaporation of the dried organic layer. **^{1}H NMR** ($CDCl_3$) δ: 7.33 (10 H, m, aromatic), 6.95 (1 H, s, benzhydryl CH), 5.52 (1 H, d, β-lactam CH, 4.2 Hz), 4.58 (1 H, d, β-lactam CH, 4.2 Hz), 4.50 (1 H, s, thiazolidine CH), 1.78 (2 H, s, NH_2), 1.64 (3 H, s, methyl), 1.27 (3 H, s, methyl). **^{13}C NMR** ($CDCl_3$) δ: 177.95 (β-lactam carbonyl), 167.20 (ester carbonyl), 139.28, 139.20, 128.63, 128.59, 128.40, 128.18, 127.66, 127.02, (aromatic C, CH), 78.33 (benzhydryl CH), 70.18 (β-lactam CH), 70.08 (β-lactam CH), 64.16 (thiazolidine C), 62.98 (thiazolidine CH), 32.09 (methyl), 26.76 (methyl). **IR** (film) υ: 3399

(w) 1774 (s), 1297.(s) cm^{-1}. **Calcd for $C_{21}H_{22}N_2O_3S$**: C, 65.94; H, 5.79; N, 7.32. **Found**: C, 65.34; H, 5.79; N, 7.98. $[\alpha]_D^{20}$ (c 0.21, $CHCl_3$): +187.

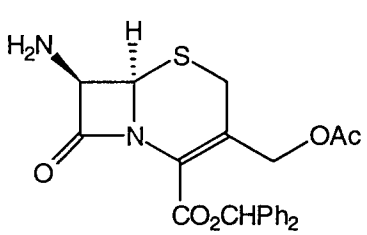
Preparation of 19



A solution of **13** (1.0 g, 3.5 mmoles), in dry methylene chloride (3 mL), was added by syringe at room temperature under nitrogen to a stirred solution of **18** (1.32 g, 3.5 mmoles) and dry pyridine (475 mg, 452 mL, 6.0 mmoles) in dry methylene chloride (6 mL). The yellow solution was stirred rapidly for 24 h and then diluted to 30 mL with methylene chloride and washed successively with 1 M hydrochloric acid (30 mL), saturated sodium bicarbonate (30 mL), saturated sodium chloride (30 mL), dried over magnesium sulfate and evaporated to a golden foam (2.0 g). This was purified by column chromatography (70% ethyl acetate-hexane) to give **19** as diastereomer A, diastereomer B and a mixture of A and B (976 mg total, 45%). One of the pure diastereomers, arbitrarily termed A, crystallized as fine needles from diethyl ether, m.p. 119-121°C. **^{1}H NMR** ($CDCl_3$) δ: (diastereomer A) 7.30 (20 H, m, aromatic), 6.92 (1 H, s, benzhydryl CH), 5.14 (1 H, d, β-lactam CH, 4.2 Hz), 5.13 (1 H, dd, benzyloxy C<u>H</u>H, 7.0, 12 Hz), 5.03 (1 H, dd, benzyloxy CH<u>H</u>, 7.0, 12.0 Hz), 4.52 (1 H, ddd, β-lactam CH, 4.2, 12.0, 10.0 Hz), 4.47 (1 H, s, thiazolidine CH), 3.41 (1 H, dd, NH, 12.0, 12.0 Hz), 3.33 (1 H, dd, benzyl C<u>H</u>H, 21.0, 14.5 Hz), 3.23 (1 H, dd, benzyl CH<u>H</u>, 21.0, 14.5 Hz), 1.51 (3 H, s, methyl), 1.22 (3 H, s, methyl). **^{1}H NMR** ($CDCl_3$) δ: (diastereomer B) 7.34 (20 H, m, aromatic), 6.91 (1 H, s, benzhydryl CH), 5.09 (1 H, dd, benzyloxy C<u>H</u>H, 8.6, 11.0 Hz), 5.06 (1 H, dd, benzyloxy CH<u>H</u>, 8.6, 11.0

Hz), 4.98 (1 H, d, β-lactam CH, 4.4 Hz), 4.76 (1 H, ddd, β-lactam CH, 4.4, 10.0, 12.0 Hz), 4.45 (1 H, s, thiazolidine CH), 3.35 (1 H, dd, NH, 11.9, 12.0 Hz), 3.24 (1 H, dd, benzyl CHH, 20.4, 14.9 Hz), 3.20 (1 H, dd, benzyl CHH, 20.4, 14.9 Hz), 1.50 (3 H, s, methyl), 1.22 (3 H, s, methyl). **^{13}C NMR** ($CDCl_3$) δ: (diastereomer A) 175.41 (d, J_{PC}=3.8 Hz, β-lactam carbonyl), 166.74 (ester carbonyl), 139.19, 139.13, 136.48, 138.47, 131.38, 130.08, 130.02, 128.72, 128.69, 128.64, 128.62, 128.60, 128.40, 128.35, 128.24, 127.99, 127.51, 127.10, 127.06 (aromatic C, CH), 78.38 (benzhydryl CH), 70.43 (β-lactam CH), 69.61 (d, J_{PC}=4.0 Hz, β-lactam CH), 66.00 (d, J_{PC}=6.9 Hz, benzyloxy CH_2), 64.67 (thiazolidine C), 61.51 (thiazolidine CH), 36.41 (d, J_{PC}=127 Hz, benzyl CH_2), 32.71 (methyl), 26.54 (methyl). **^{13}C NMR** ($CDCl_3$) δ: (diastereomer B) 175.00 (s, β-lactam carbonyl), 166.77 (ester carbonyl), 139.18, 139.13, 136.33, 136.27, 131.89, 131.80, 130.03, 129.97, 128.78, 128.76, 128.63, 128.46, 128.35, 128.27, 128.01, 127.86, 127.58, 127.06, (aromatic C, CH), 78.45 (benzhydryl CH), 70.40 (β-lactam CH), 69.69 (d, J_{PC}=4.0 Hz, β-lactam CH), 65.91 (d, J_{PC}=7.0 Hz, benzyloxy CH_2), 64.72 (thiazolidine C), 61.80 (thiazolidine CH), 36.44 (d, J_{PC}=127 Hz, benzyl CH_2), 32.80 (methyl), 26.56 (methyl). **^{31}P NMR** {^{1}H} δ: 28.63, 28.27. **IR** (KBr) υ (cm^{-1}): (diastereomer A) 3344 (m), 1784 (s), 1744 (s), 1206 (s), 1021 (s). **Calcd for $C_{35}H_{35}N_2O_5SP$**: C, 67.08; H, 5.63; N, 4.47. **Found**: C, 66.97; H, 5.69; N, 4.58. **Mass spectrum** (CI): decomposes. $[\alpha]_D^{20}$ (c 0.18, $CHCl_3$): +129.

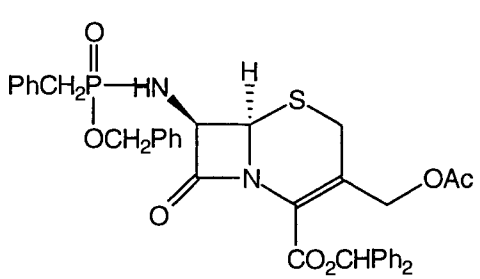
Preparation of 20



A solution of diphenyldiazomethane (1.0 g, 5.1 mmoles) in dry methylene chloride (10 mL) was added to a stirred suspension of 7-

aminocephalosporanic acid (1.36 g, 5.0 mmoles) in dry methylene chloride (25 mL) and acetonitrile (35 mL). The mixture was heated to reflux for 24 h and the resulting purple solution was concentrated to a purple oil, washed with saturated sodium bicarbonate, dried over magnesium sulfate and evaporated to a yellow oil. Column chromatography (50 % ethyl acetate-hexane) gave **20**. **^{1}H NMR** ($CDCl_3$) δ: 7.33 (10 H, m, aromatic), 6.98 (1 H, s, benzhydryl CH), 4.97 (1 H, d, acetate C<u>H</u>H, 14.6 Hz), 4.92 (1 H, d, β-lactam CH, 5 Hz), 4.80 (1 H, d, β-lactam CH, 5 Hz), 4.79 (1 H, d, acetate CH<u>H</u>, 14.6 Hz), 3.56 (1 H, d, thiazine C<u>H</u>H, 18.6 Hz), 3.39 (1 H, d, thiazine CH<u>H</u>, 18.6 Hz), 2.00 (3 H, s, acetyl methyl), 1.78 (2 H, s, NH_2). **^{13}C NMR** ($CDCl_3$) δ: 170.42 (acetyl carbonyl), 168.92 (β-lactam carbonyl), 161.11 (ester carbonyl), 139.41, 139.20, 128.54, 128.48, 128.20, 128.06, 127.83, 127.19, 126.21, 125.62, (aromatic C, CH, thiazine C), 79.69 (benzhydryl CH), 63.88 (β-lactam CH), 63.21 (C3' CH_2), 58.93 (β-lactam CH), 26.26 (thiazine CH_2), 20.62 (acetyl CH_3). **IR** (film) υ: 3404 (m), 1778 (s), 1736 (s), 1380 (s), 1229 (s) cm^{-1}. **Calcd for $C_{23}H_{22}N_2O_5S$**: C, 63.00; H, 5.06; N, 6.39. **Found**: C, 63.50; H, 5.29; N, 6.23. **Mass spectrum (CI):** decomposes. **$[\alpha]_D$** (c 0.23, $CHCl_3$): -28.

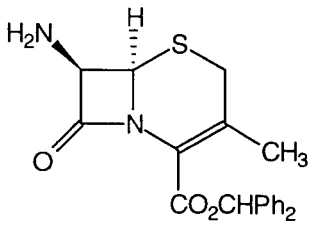
Preparation of 21



At room temperature under nitrogen a solution of **13** (295 mg, 1.05 mmoles) in dry methylene chloride (1 mL) was added by syringe to a solution of **20** (448 mg, 1.02 mmoles) and dry pyridine (160 μL, 168 mg, 2.12 mmoles) in dry methylene chloride (2 mL). The reaction mixture was

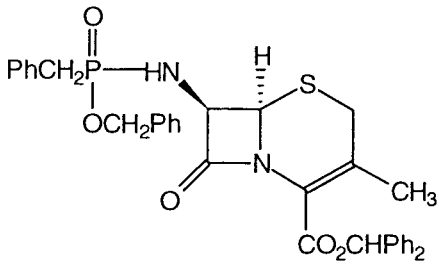
stirred for 24 h, diluted with methylene chloride (15 mL) and washed with 1 M hydrochloric acid (15 mL) and saturated sodium bicarbonate (15 mL). The organic layer was dried over magnesium sulfate and evaporated to a yellow oil. Column chromatography (80% ethyl acetate-hexane) afforded **21** (237 mg, 34%) as a mixture of diastereomers. **^{1}H NMR** ($CDCl_3$) δ: 7.30 (20 H, m, aromatic), 6.94 (1 H, s, benzhydryl CH (one diastereomer)), 6.92 (1 H, s, benzhydryl CH (one diastereomer)), 5.08 (2 H, m, benzyloxy CH_2), 4.99 (1 H, d, acetate C<u>H</u>H, 13 Hz), 4.89 (1 H, ddd, β-lactam CH; 5.0, 12.0, 10 Hz (one diastereomer)), 4.75 (1 H, d, acetate CH<u>H</u>, 13 Hz), 4.70 (1 H, d, β-lactam CH, 5.0 Hz (one diastereomer)), 4.69 (1 H, d, β-lactam CH, 5.0 Hz (one diastereomer)), 3.35 (5 H, m, NH, benzyl CH_2, thiazine CH_2), 2.02 (3 H, s, acetyl methyl). **^{13}C NMR** ($CDCl_3$) δ: 170.38 (acetyl carbonyl), 166.44 (β-lactam carbonyl), 161.10, 160.84, (ester carbonyl), 139.35, 139.16, 136.34, 136.27, 131.57, 131.48, 130.17, 130.11, 128.74, 128.60, 128.57, 128.50, 128.31, 128.19, 128.11, 127.82, 127.71, 127.13, 126.84, 126.62, 126.57, 125,86, (aromatic C, CH, thiazine C), 79.76, 79.67, (benzhydryl CH), 65.93, 65.86, (benzyloxy CH_2), 63.84, 63.20, 63.06, 62.33, 61.82, 58.90, 58.47, (2 β-lactam CH, C3' CH_2), 37.09, 36.93, 35.83, 35.66, (benzyl CH_2), 26.38, 26.24, (acetyl CH_3), 20.61 (thiazine CH_2). **^{31}P NMR** {^{1}H} δ: 28.61, 28.33. **IR** (film) υ: 3169 (m), 1785 (s), 1736 (s), 1228 (s), 1024 (s) cm^{-1}. **Calcd for $C_{37}H_{35}N_2O_7SP•0.5\ H_2O$**: C, 64.24; H, 5.24; N, 4.05. **Found**: C, 63.95; H, 5.17; N, 4.42. **Mass spectrum (CI):** decomposes. $[\alpha]_D^{20}$ (c 0.31, $CHCl_3$): +16.

Preparation of 22



At room temperature under nitrogen a solution of diphenyldiazomethane (1.0 g, 5.2 mmoles) in dry methylene chloride (10 mL) was added to a stirred suspension of 7-aminodesacetoxy-cephalosporanic acid (1.07 g, 5.0 mmoles) in dry methylene chloride (10 mL) and acetonitrile (35 mL). This purple mixture was heated to reflux, stirred overnight and the solvent was evaporated. The yellow oil was purified by column chromatography (70% ethyl acetate-hexane) to give **20**. **^{1}H NMR** ($CDCl_3$) δ: 7.33 (10 H, m, aromatic), 6.95 (1 H, s, benzhydryl CH), 4.93 (1 H, d, β-lactam CH, 4.3 Hz), 4.75 (1 H, d, β-lactam CH, 4.3 Hz), 3.47 (1 H, d, thiazine C<u>H</u>H, 16.7 Hz), 3.25 (1 H, d, thiazine CH<u>H</u>, 16.7 Hz), 2.30 (2 H, s, NH_2), 2.12 (3 H, s, methyl). **^{13}C NMR** ($CDCl_3$) δ: 168.83 (β-lactam carbonyl), 161.79 (ester carbonyl), 139.89, 139.76, 132.07, 128.53, 128.45, 127.99, 127.62, 127.22, (aromatic C, CH, thiazine C), 78.97 (benzhydryl CH), 63.47 (C6 β-lactam CH), 58.77 (C7 β-lactam CH), 30.11 (thiazine CH_2), 20.25 (C3 CH_3). **IR** (film) υ: 3400 (m), 1773 (s), 1720 (s) cm^{-1}. **Calcd for $C_{21}H_{20}N_2O_3S$**: C, 66.29; H, 5.30; N, 7.36 **Found**: C, 65.68; H, 5.34; N, 7.14. **Mass spectrum** (CI): 381 (M+), 167. $[\alpha]_D$ (c 0.20, $CHCl_3$): +49.

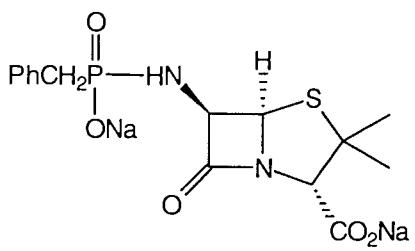
Preparation of 23



At room temperature under nitrogen a solution of **13** (295 mg, 1.05 mmoles) in dry methylene chloride (1 mL) was added by syringe to a solution of **22** (386 mg, 1.05 mmoles) and dry pyridine (160 µL, 168 mg, 2.12 mmoles) in dry methylene chloride (2 mL). The reaction mixture was stirred for 24 h, diluted with methylene chloride (15 mL), washed with 1 M hydrochloric acid (12 mL) and saturated sodium bicarbonate (12 mL), dried over magnesium sulfate and evaporated to a yellow oil. Column chromatography (80% ethyl acetate-hexane) gave **23** (330 mg, 51%) as a mixture of diastereomers. **^{1}H NMR** ($CDCl_3$) δ: 7.30 (20 H, m, aromatic), 6.90 (1 H, s, benzhydryl CH (one diastereomer)), 6.89 (1 H, s, benzhydryl CH (one diastereomer)), 5.09 (3 H, m, benzyloxy CH_2, β-lactam CH (one diastereomer)), 4.83 (1 H, ddd, β-lactam CH, 4.0, 12.0, 10.0 Hz) 4.69 (1 H, d, β-lactam CH, 4.0 Hz (one diastereomer)), 4.66 (1 H, d, β-lactam CH 4.0 Hz (one diastereomer)), 3.30 (5 H, m, benzyl CH_2, thiazine CH_2, NH), 2.09 (3 H, s, methyl). **^{13}C NMR** ($CDCl_3$) δ: 166.85 (d, J_{PC}=4.73 Hz, β-lactam carbonyl (one diastereomer)), 166.38 (d, J_{PC}=5.94 Hz, β-lactam carbonyl (one diastereomer)), 161.54 (ester carbonyl), 139.88, 139.85, 136.53, 133.77, 133.74, 130.27, 130.24, 130.21, 130.18, 128.74, 128.68, 128.60, 128.50, 128.26, 128.03, 127.99, 127.83, 127.81, 127.52, 127.18, 127.07, 122.77, (aromatic C, CH, thiazine C), 79.02 (benzhydryl CH), 65.98, 65.92, 65.85, (m, benzyloxy CH_2), 61.97 (C6 β-lactam CH (one diastereomer)), 61.49 (C6 β-lactam CH (one diastereomer)), 58.53, 58.49, 58.46, (m, C7 β-lactam CH), 36.38 (d, J_{PC}=126.6, benzyl CH_2 (one

diastereomer)), 36.19 (d, J_{PC}=127.4 Hz, benzyl CH_2 (one diastereomer)), 30.26 (C3' CH_3), 20.22 (thiazine CH_2). **^{31}P NMR** {^{1}H} δ: 28.54, 28.75. **IR** (film) υ: 3167 (m), 1780 (s), 1723 (s), 1219 (s) cm^{-1}. **Calcd for $C_{28}H_{27}N_2O_5SP•0.5\ H_2O$**: C, 66.33; H, 5.41; N, 4.42. **Found**: C, 66.35; H, 5.39; N, 4.38. **Mass spectrum** (CI): decomposes. $[\alpha]_D^{20}$ (c 0.25, $CHCl_3$): +32.

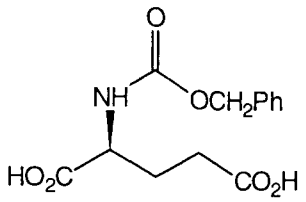
Preparation of 24



A solution of sodium bicarbonate (21.3 mg, 0.25 mmole) in water (3 mL) was added with rapid stirring to a solution of **19** (76 mg, 0.12 mmole) in tetrahydrofuran (12 mL). Ten percent palladium on carbon (150 mg) was then added and the three-necked reaction vessel was sealed with rubber septa and a balloon. The system was purged twice with nitrogen, twice with hydrogen, and hydrogen was added to inflate the balloon fully. The mixture was stirred for 1 h, the pressure was released, and the suspension was filtered through a prewashed Celite pad (3 cm) and washed repeatedly with tetrahydrofuran and water (5 mL aliquots). The combined filtrates were concentrated at 30° C to remove most of the tetrahydrofuran and then lyophilized to give a black powder (70 mg). This was dissolved in ice cold water (6 mL) and washed with ice cold ethyl acetate (2 x 6 mL). The aqueous portion was lyophilized to give **24** as a grey powder (40 mg, 80%). **^{1}H NMR** (D_2O) δ: 7.28 (5 H, m, aromatic), 5.17 (1 H, d, β-lactam CH, 4.0 Hz), 4.60 (1 H, dd, β-lactam CH 10.0, 4.0 Hz), 4.10 (1 H, s, thiazolidine CH), 3.02 (1 H, dd, benzyl C<u>H</u>H, 19.5, 14.0 Hz), 2.95 (1 H, dd, benzyl CH<u>H</u>, 19.5, 14.0 Hz), 1.44 (3 H, s,

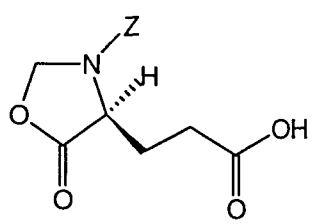
methyl), 1.53 (3 H, s, methyl). **IR** KBr: 3383 (s), 1762 (s), 1603 (s), 1178 (s) cm^{-1}.

Preparation of 25



L-Glutamic acid (5.0 g, 34 mmoles) was slowly added at room temperature to a suspension of sodium bicarbonate (10.7 g, 127 mmoles) in water (65 mL). After the effervescence subsided, benzyl chloroformate (7.0 mL, 8.4 g, 47 mmoles) was added dropwise, with stirring, during 15 min. Stirring was continued for 3 h, and ether (30 mL) was then added. The aqueous layer was separated, acidified to pH 3 with 6 M hydrochloric acid, and extracted with ethyl acetate (2 x 80 mL). The organic extract was washed with saturated sodium chloride (100 mL), dried over anhydrous magnesium sulfate and recrystallized from ethyl acetate and hexane to give **25** as white crystals (3.79 g, 40%). **^{1}H NMR** (acetone d_6) δ: 7.33 (5 H, s, aromatic), 6.65 (1 H, d, NH, 8.3 Hz,), 5.10 (2 H, s, benzyloxy CH_2), 4.30 (1 H, dt, α CH, 8.3, 4.7 Hz), 2.5 (2 H, m, alkyl CH_2), 2.20 (2 H, m, alkyl CH_2).

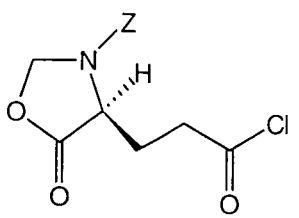
Preparation of 26



Paraformaldehyde (2.0 g, 66 mmoles) and p-toluenesulfonic acid monohydrate (200 mg, 1.1 mmoles) were added to a stirred solution of **25** (2.81 g, 10 mmoles) in toluene (150 mL) and the mixture was heated to reflux in a flask fitted with a Dean-Stark trap. The reaction was

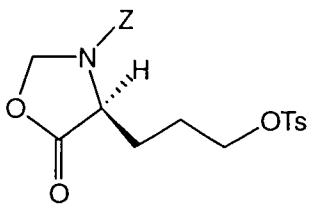
stopped after 1 h, when no further water was collected. The solution was cooled to room temperature and extracted with saturated sodium bicarbonate (2 x 50 mL). The aqueous extract was acidified to pH 4 with 5 M hydrochloric acid, and extracted with ethyl acetate (2 x 100 mL). The organic layer was dried over anhydrous magnesium sulfate and evaporated to afford **26** as a colourless oil (2.0 g, 68%). **^{1}H NMR** ($CDCl_3$) δ: 7.38 (5 H, s, aromatic), 5.56 (1 H, d, oxazolidinone C<u>H</u>H, 3.0 Hz), 5.28 (1 H, d, oxazolidinone CH<u>H</u>, 3.0 Hz), 5.20 (2 H, s, benzyloxy CH_2), 4.5 (1 H, t, α CH, 5.0 Hz), 2.28 (4 H, m, alkyl $(CH_2)_2$).

Preparation of 27



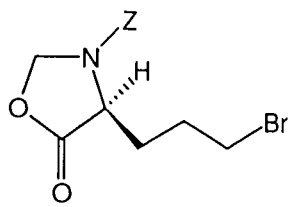
Oxalyl chloride (2.5 mL, 3.7 g, 29 mmoles) was injected into a solution of **26** (2.0 g, 6.8 mmoles) and dimethyl formamide (1 drop) in dry toluene (100 mL). The solution was stirred for 2.5 h, additional oxalyl chloride (1.0 mL, 1.54 g, 11.7 mmoles) was added and the pale yellow solution was heated to reflux for 1 h, then cooled to room temperature. The solvent was evaporated under reduced pressure. The product **27** crystallized as yellow needles from ethyl acetate (8 mL) and hexane (8 mL). The crystals were collected, washed with hexane and air dried (1.41 g, 67%). **^{1}H NMR** ($CDCl_3$) δ: 7.4 (5 H, s, aromatic), 5.6 (1 H, d, oxazolidinone C<u>H</u>H, 2.5 Hz), 5.2 (1 H, d, oxazolidinone CH<u>H</u>, 2.5 Hz), 5.2 (2 H, s, benzyloxy CH_2), 4.4 (1 H, t, α CH, 7.0 Hz), 3.1 (2 H, t, alkyl CH_2COCl, 7.0 Hz), 2.3 (2 H, m, alkyl CH_2).

Preparation of 28



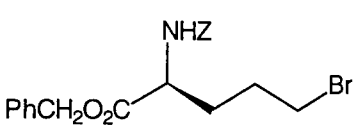
Rapid addition of sodium borohydride (257 mg, 6.80 mmoles) in water (2 mL) to a solution of **27** (1.41 g, 4.53 mmol) in dimethoxyethane (5 mL) at -8° C led to a violent evolution of gas. Water (100 mL) was immediately added and the mixture was stirred rapidly. The product was extracted with ethyl acetate (2 x 75 mL), dried over anhydrous magnesium sulfate and evaporated under reduced pressure to a yellow oil (1.06 g). The residue was dissolved in chloroform (3 mL), and pyridine (421 μL, 5.6 mmoles) and p-toluenesulfonyl chloride (800 mg, 4.2 mmoles) were added with stirring at 0° C. After 2 h diethyl ether (10 mL) and water (3 mL) were added and the organic layer was washed with 0.3 M hydrochloric acid (5 mL) and saturated sodium bicarbonate (5 mL), dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give the crude tosylate (1.3 g). The material was purified by column chromatography (40% ethyl acetate-hexane) to give **28** (475 mg, 24%) as a pale yellow oil. **^{1}H NMR** ($CDCl_3$) δ: 7.80 (2 H, d, aromatic, 8 Hz), 7.30 (7 H, m, aromatic), 5.50 (1 H, d, oxazolidinone C<u>H</u>H, 3 Hz), 5.22 (3 H, m, benzyloxy CH_2, oxazolidinone CH<u>H</u>), 4.33 (1 H, m, α CH), 4.00 (2 H, m, alkyl CH_2O), 2.50 (3 H, s, tosyl CH_3), 1.81 (4 H, m, alkyl $(CH_2)_2$).

Preparation of 29



Tetrabutylammonium bromide (170 mg, 0.54 mmole) was added to a solution of **28** (20 mg, 0.046 mmole) in methylene chloride (1.5 mL) and the solution was heated to reflux for 24 h. The solution was then cooled, dilluted with methylene chloride (4 mL) and water (4 mL) and the organic layer was separated, washed with saturated sodium chloride (5 mL), dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give 72 mg of material. This was purified by column chromatography (40% ethyl acetate-hexane) to yield **29** (10 mg, 63%). **^{1}H NMR** ($CDCl_3$) δ: 7.3 (5 H, s, aromatic), 5.50 (1 H, d, oxazolidinone C<u>H</u>H, 3 Hz), 5.25 (3 H, m, benzyloxy CH_2, oxazolidinone CH<u>H</u>), 4.33 (1 H, m, α CH), 3.50 (2 H, m, alkyl CH_2Br), 2.00 (4 H, m, alkyl $(CH_2)_2$).

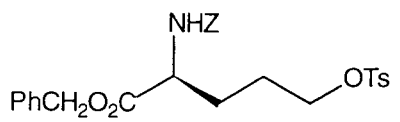
Preparation of 30



A solution of **11** (230 mg, 0.88 mmol) in dry tetrahydrofuran (1 mL) was slowly added to a suspension of sodium hydride (23 mg, 0.96 mmole) in dry tetrahydrofuran (3 mL) at 0° C and the mixture was stirred for 45 min, then withdrawn into a syringe and slowly added at -78° C to a solution of **29** (300 mg, 0.88 mmole) in dry tetrahydrofuran (1 mL) at -78° C. Water (3 mL) was added after 45 min and the products were extracted into methylene chloride (5 mL). The organic layer was washed with saturated sodium chloride (5 mL), dried over anhydrous magnesium sulfate and evaporated under reduced pressure to leave 380 mg of a

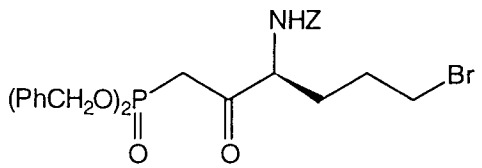
yellow oil. Chromatography on silica gel (30% ethyl acetate - hexane) gave one major product, **30** (80 mg, 22%). **^{1}H NMR** ($CDCl_3$) δ: 7.30 (10 H, m, aromatic), 5.33 (1 H, d, NH, 8.0 Hz), 5.22 (1 H, d, benzyl ester C<u>H</u>H, 13.0 Hz), 5.12 (1 H, d, benzyl ester CH<u>H</u>, 13.0 Hz), 5.10 (2 H, s, benzyloxycarbonyl CH_2), 4.42 (1 H, m, α CH), 3.50 (2 H, t, alkyl CH_2Br, 7.0 Hz), 1.85 (4 H, m, alkyl $(CH_2)_2$).

Preparation of 30a



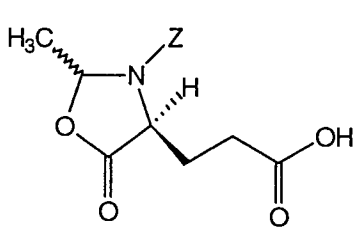
n-Butyllithium (160 µL of a 2.5 M solution in hexane, 0.400 mmole) was added under nitrogen at -60° C to a solution of **11** (100 mg, 0.38 mmol) in dry tetrahydrofuran (1 mL). The mixture was stirred for 45 min and **28** (173 mg, 0.400 mmole) in dry tetrahydrofuran (800 µL) was then added. Stirring was continued for 30 min, with warming to -15° C, and then for 1 h, with warming to 20° C. Methylene chloride (12 mL) was added and the organic layer was collected and washed with water (12 mL), saturated sodium chloride (12 mL), dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give 234 mg of an oil. This was partially purified by column chromatography (40% ethyl acetate-hexane) to yield **30a** (35 mg, 17%). **^{1}H NMR** ($CDCl_3$) δ: 7.77 (2 H, d, aromatic, 9 Hz), 7.32 (7 H, m, aromatic), 5.29 (1 H, d, NH, 8 Hz), 5.16 (2 H, s, benzyl ester CH_2), 5.09 (2 H, s, benzyloxycarbonyl CH_2), 4.35 (1 H, m, α CH), 4.00 (2 H, m, alkyl CH_2OTs), 2.41 (3 H, s, tosyl methyl), 1.80 (4 H, m, alkyl $(CH_2)_2$).

Preparation of 30b



n-Butyl lithium (60 μL of a 2.5 M solution in hexane, 0.15 mmole), was injected at -78° C into a solution of dibenzyl methylphosphonate (39 mg, 0.14 mmole) in dry tetrahydrofuran (0.5 mL). The mixture was stirred for 1.5 h and **34** (50 mg, 0.14 mmole) in dry tetrahydrofuran (0.3 mL) was then added during 5 min. The cooling bath was removed, and the mixture was stirred for 7 h and diluted with tetrahydrofuran (2 mL) and 0.5 M hydrochloric acid (1 mL). Saturated sodium chloride (2 mL) was added and the products were extracted with ethyl acetate (2 x 5 mL). The organic layer was dried over anhydrous magnesium sulfate and evaporated under reduced pressure to 87 mg of a yellow oil. This was purified by column chromatography (60% ethyl acetate-hexane) to yield **30b** (14 mg, 17%). **^{1}H NMR** ($CDCl_3$) δ: 7.37 (15 H, s, aromatic), 5.80 (1 H, d, NH, 8.3 Hz), 5.10 (2 H, s, benzyloxycarbonyl CH_2), 5.03 (4 H, m, benzyloxy CH_2), 4.40 (1 H, m, α CH), 3.47 (2 H, m, alkyl CH_2Br), 3.30 (1 H, dd, alkyl PC<u>H</u>HCO, 23.3, 13.3 Hz), 3.07 (1 H, dd, alkyl PCH<u>H</u>CO, 23.3, 13.3 Hz), 1.80 (4 H, m, alkyl $(CH_2)_2$).

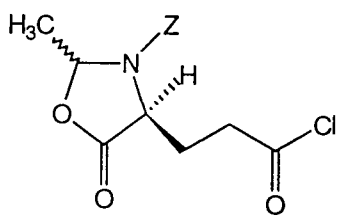
Preparation of 31



Compound **25** (373 mg, 1.33 mmole), acetaldehyde (155 μL, 153 mg, 1.15 mmole) and para-toluenesulfonic acid monohydrate (20 mg, 0.11 mmole) were suspended in dichloroethane (5 mL) and heated to

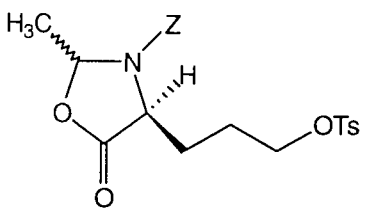
reflux using a Dean-Stark trap. After 1 h 2 mL of the solvent in the Dean-Stark trap were removed and 2 mL of fresh solvent were added to the reaction mixture. This process was repeated five times and more acetaldehyde (300 μL, 300 mg, 5.2 mmoles) was then added. The solution was refluxed 12 h, acetaldehyde (200 μL, 200 mg, 3.47 mmol) was added and the replacement of the solvent was repeated for 9 h. The mixture was then cooled, evaporated under reduced pressure, and the product was purified by column chromatography (60% toluene-ether) to give **31** (122 mg, 30%). **^{1}H NMR** ($CDCl_3$) δ: 7.30 (5 H, s, aromatic), 5.83 (1 H, q, oxazolidinone NCHO, 5.7 Hz), 5.20 (2 H, s, benzyloxy CH_2), 4.40 (1 H, t, α CH, 5.7 Hz), 2.30 (4 H, m, alkyl $(CH_2)_2$), 1.60 (3 H, d, methyl, 5.0 Hz).

Preparation of 32



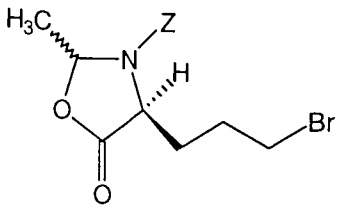
Oxalyl chloride (700 μL, 590 mg, 8.07 mmoles) was injected into a solution of **31** (590 mg, 1.92 mmoles) and dimethyl formamide (1 drop) in dry toluene (25 mL). The solution was stirred for 1.5 h, heated to reflux for 0.5 h and cooled to room temperature. The solvent was evaporated under reduced pressure to give a golden oil (522 mg, 84%). **^{1}H NMR** ($CDCl_3$) δ:7.30 (5 H, m, aromatic), 5.83 (1 H, q, oxazolidinone NCHO, 5.5 Hz), 5.22 (2 H, s, benzyloxy CH_2), 4.33 (1 H, t, α CH, 6.7 Hz), 3.17 (2 H, t, CH_2COCl, 6.7 Hz),. 2.32 (2 H, m, alkyl CH_2), 1.60 (3 H, d, methyl, 5.5 Hz).

Preparation of 33



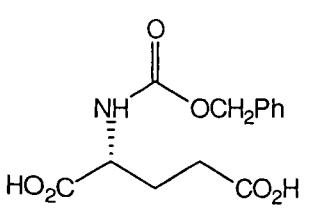
Sodium borohydride (91 mg, 2.4 mmoles), in water (1 mL), was added, with rapid stirring at -10° C, to a solution of **32** (522 mg, 1.6 mmoles) in dimethoxyethane (2 mL). After 3 sec, water (20 mL) was added and stirring was continued for 10 min. The product was extracted into methylene chloride (3 x 20 mL) and this extract was washed with saturated sodium chloride (30 mL), dried over anhydrous magnesium sulfate and evaporated to give a yellow oil (410 mg). This was dissolved in chloroform (2 mL), the solution cooled to 0° C, and pyridine (200 μL, 2.65 mmoles) and p-toluenesulfonic chloride (370 mg, 1.96 mmol) were added. The mixture was stirred at 0° C for 1 h, and at 20° C for 2h. Methylene chloride (20 mL) was added and the solution was washed with 0.25 M hydrochloric acid (10 mL), saturated sodium bicarbonate (10 mL) and saturated sodium chloride (10 mL), dried over anhydrous magnesium sulfate and evaporated to a yellow oil (626 mg). Column chromatography (40% ethyl acetate-hexane) gave **33** (330 mg, 46%). **^{1}H NMR** ($CDCl_3$) δ: 7.80 (2 H, d, aromatic, 8.3 Hz), 7.37 (7 H, m, aromatic), 5.80 (1 H, q, oxazolidinone NCHO, 5.5 Hz), 5.20 (2 H, s, benzyloxy CH_2), 4.28 (1 H, m, α CH), 4.03 (2 H, m, alkyl CH_2OTs), 2.47 (3 H, s, tosyl methyl), 1.90 (4 H, m, alkyl $(CH_2)_2$), 1.60 (3 H, d, methyl, 5.5 Hz).

Preparation of 34



Tetrabutylammonium bromide (504 mg, 1.57 mmoles) was added to a solution of **33** (70 mg, 0.157 mmole), in dry methylene chloride (5 mL), and the solution was heated to reflux for 24 h. Water (5 mL) was then added and the organic layer was separated and washed with saturated sodium chloride (3 mL), dried over anhydrous magnesium sulfate and evaporated to 215 mg of a yellow oil. Column chromatography (35% ethyl acetate-hexane) afforded **34** (50 mg, 89%). **^{1}H NMR** ($CDCl_3$) δ: 7.36 (5 H, s, aromatic), 5.83 (1 H, q, oxazolidinone NCHO, 5.5 Hz), 5.18 (2 H, s, benzyloxy CH_2), 4.33 (1 H, m, α CH), 3.53 (2 H, m, alkyl CH_2Br), 2.07 (4 H, m, alkyl $(CH_2)_2$), 1.58 (3 H, d, methyl, 5.5 Hz).

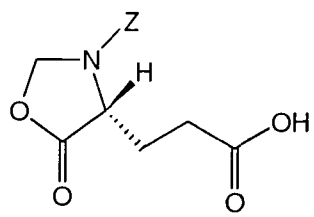
Preparation of 35



D-Glutamic acid (5.0 g, 34 mmoles) was slowly added at room temperature to a suspension of sodium bicarbonate (10.7 g, 127 mmoles) in water (65 mL). After the effervescence had subsided, benzyl chloroformate (7.0 mL, 8.4 g, 47 mmoles) was added dropwise, with stirring, during 15 min. Stirring was continued for 3 h, and ether (50 mL) was then added. The aqueous layer was separated, acidified to pH 4 with 5 M hydrochloric acid, and extracted with ethyl acetate (2 x 100 mL). The organic extract was washed with saturated sodium chloride (100 mL),

dried over anhydrous magnesium sulfate and evaporated to give **35** as a foam (7.74 g, 81%). **^{1}H NMR** (acetone d_6) δ: 10.40 (2 H, s, $(CO_2H)_2$), 7.33 (5 H, m, aromatic), 6.65 (1 H, d, NH, 8.3 Hz,), 5.09 (2 H, s, benzyloxy CH_2), 4.30 (1 H, dt, α CH, 8.3, 4.7 Hz), 2.5 (2 H, m, alkyl CH_2), 2.20 (2 H, m, alkyl CH_2). **^{13}C NMR** (acetone d_6) δ: 174.21 (acid carbonyl), 173.67 (acid carbonyl), 157.15 (urethane carbonyl), 138.07, 129.15, 128.59, (aromatic C, CH), 66.80 (benzyl CH_2), 54.10 (CH), 30.46 (alkyl CH_2), 27.67 (alkyl CH_2). **IR** (KBr) υ: 3305 (s), 3032 (br), 1712 (s), 1549 (s) cm^{-1}. **Calcd for $C_{13}H_{15}NO_6$**: C, 55.51; H, 5.38; N, 4.98 **Found**: C, 55.12; H, 5.48; N, 4.94. **Mass spectrum:** decomposes. $[\alpha]_D^{20}$ (c 0.7, acetone): +15.3.

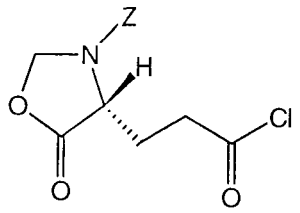
Preparation of 36



Paraformaldehyde (6.0 g, 200 mmoles) and p-toluenesulfonic acid monohydrate (600 mg, 3.2 mmoles) were added to a stirred solution of **35** (9.0 g, 32 mmoles) in toluene (300 mL) and the mixture was heated to reflux in a flask fitted with a Dean-Stark trap. The reaction was stopped after 2 h, when no further water was collected. The solution was cooled to room temperature and extracted with saturated sodium bicarbonate (3 x 150 mL). The aqueous extract was acidified to pH 3 with 1 M hydrochloric acid and extracted with methylene chloride (3 x 150 mL). The organic layer was dried over anhydrous magnesium sulfate and evaporated to afford **36** as a colourless viscous oil (6.2 g, 70%). **^{1}H NMR** ($CDCl_3$) δ: 7.38 (5 H, m, aromatic), 5.56 (1 H, d, oxazolidinone C<u>H</u>H, 4.0 Hz), 5.24 (1 H, d, oxazolidinone CH<u>H</u>, 4.0 Hz), 5.20 (2 H, s, benzyloxy CH_2), 4.42 (1 H, m, α CH), 2.53 (2 H, m, alkyl CH_2), 2.28 (2 H, m, alkyl CH_2).**^{13}C NMR** ($CDCl_3$) δ: 174.01 (acid carbonyl), 172.90 (oxazolidone

carbonyl), 154.00 (urethane carbonyl), 137.04, 129.20, 128.85, 128.67, (aromatic C, CH), 78.62 (oxazolidone CH_2), 67.93 (benzyl CH_2), 54.81 (α CH), 29.42 (alkyl CH_2), 26.71 (alkyl CH_2). **IR** (KBr) υ: 3132 (br), 3034 (br), 1794 (s), 1713 (s) cm^{-1}. **Calcd for $C_{14}H_{15}NO_6$**: C, 57.34; H, 5.16; N, 4.78 **Found**: C, 56.96; H, 5.54; N, 4.47. **Mass spectrum:** decomposes. $[\alpha]_D^{20}$ (c 1.4, $CHCl_3$): -85.4.

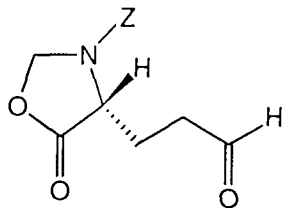
Preparation of 37



Oxalyl chloride (3.5 mL, 5.2 g, 41 mmoles) was injected into a solution of **36** (2.71 g, 9.0 mmoles) and dimethyl formamide (3 drops) in dry toluene (400 mL). The solution was stirred for 3 h, additional oxalyl chloride (1.0 mL, 1.54 g, 11.7 mmoles) was added, and the pale yellow solution was heated to reflux for 1 h, cooled to room temperature and evaporated under reduced pressure. The product **37** crystallized as yellow needles from ethyl acetate (10 mL) and hexane (10 mL). The crystals were collected and air dried (1.8 g, 62%), m.p. 62-63°C. **^{1}H NMR** ($CDCl_3$) δ: 7.39 (5 H, s, aromatic), 5.55 (1 H, d, oxazolidinone C<u>H</u>H, 2.5 Hz), 5.24 (1 H, d, oxazolidinone CH<u>H</u>, 2.5 Hz), 5.21 (2 H, s, benzyloxy CH_2), 4.38 (1 H, t, α CH, 7.0 Hz), 3.09 (2 H, t, alkyl CH_2COCl, 7.0 Hz), 2.32 (2 H, m, alkyl CH_2). **^{13}C NMR** ($CDCl_3$) δ: 172.71 (carbonyl), 171.14 (carbonyl), 153.06 (urethane carbonyl), 135.01, 128.69, 128.63, 128.57, 128.39, 128.25, (aromatic C, CH), 77.77 (oxazolidinone CH_2), 68.33 (benzyloxy CH_2), 53.37 (α CH), 42.20 (alkyl CH_2), 26.21 (alkyl CH_2). **IR** (KBr) υ: 1783 (s), 1688 (s) cm^{-1}. **Calcd for $C_{14}H_{14}NO_5Cl$**: C, 53.94; H, 4.53;

N, 4.49 **Found**: C, 53.94; H, 4.61; N, 4.50. **Mass spectrum** (CI) m/e: 312, 294, 250 (-COCl). $[\alpha]_D^{20}$ (c 0.71, $CHCl_3$): -86.8.

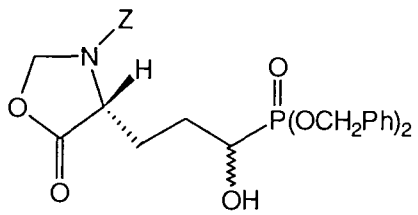
Preparation of 38



A 1 M solution of lithium tri-tert-butoxy aluminum hydride in tetrahydrofuran (16 mL) was added by syringe, under nitrogen at -75° C, during 45 min to a rapidly stirred solution of **37** (5.0 g, 16 mmoles) in dry tetrahydrofuran (40 mL). The mixture was stirred for 1 h and the cooling bath was then removed and the solution allowed to warm to room temperature. The reaction was quenched by pouring into a mixture of ice-water (30 mL) and ethyl acetate (70 mL). This was stirred for 5 min and extracted with ethyl acetate (2 x 70 mL), using centrifugation to remove insoluble emulsified salts. The ethyl acetate extracts were combined, dried over anhydrous magnesium sulfate and evaporated to give the aldehyde **38** as a yellow oil (2.5 g, 50%). **^{1}H NMR** ($CDCl_3$) δ: 9.70 (1 H, s, aldehyde CH), 7.38 (5 H, s, aromatic), 5.54 (1 H, d, oxazolidinone C<u>H</u>H, 5.0 Hz), 5.21 (1 H, d, oxazolidinone CH<u>H</u>, 5.0 Hz), 5.20 (2 H, s, benzyloxy CH_2), 4.39 (1 H, t, α CH, 7.0 Hz), 2.63 (2 H, m, alkyl C<u>H</u>$_2$CHO), 2.30 (2 H, m, alkyl CH_2). **^{13}C NMR** ($CDCl_3$) δ: 199.82 (aldehyde carbonyl), 171.59 (carbonyl), 152.96 (urethane carbonyl), 135.17, 128.66, 128.57, 128.31, 128.24, (aromatic C, CH), 77.67 (oxazolidinone CH_2), 68.11 (benzyloxy CH_2), 53.84 (α CH), 38.76 (alkyl CH_2), 23.33 (alkyl CH_2). **IR**

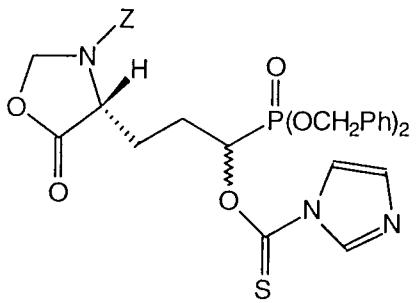
($CHCl_3$) υ: 1802 (s), 1719 (s) cm^{-1}. **Mass spectrum** (CI) m/e: 278 (M+), 250 (-CO), 234 (-CH_3CHO).

Preparation of 39



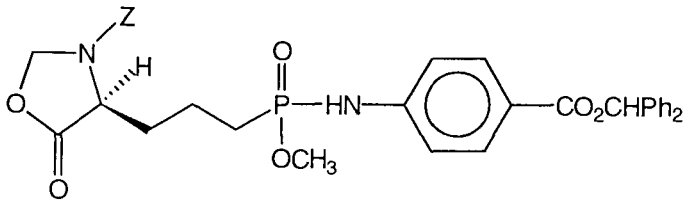
Titanium tetraisopropoxide (700 mL, 674 mg, 2.4 mmoles) was injected into a solution of **38** (1.33 g, 2.5 mmoles) and dibenzyl phosphite (3.67 g, 14 mmoles) in dry toluene (8 mL). The solution was stirred overnight at room temperature, the solvent was evaporated, the product was purified by column chromatography (80% ethyl acetate-hexane) to yield the oil **39** (2.0 g, 77%) as a mixture of diastereomers. **^{1}H NMR** ($CDCl_3$) δ: 7.36 (15 H, s, aromatic), 5.52 (1 H, d, oxazolidinone C<u>H</u>H, 5.0 Hz), 5.10 (7 H, m, benzyloxy CH_2, two phosphonobenzyloxy CH_2, oxazolidinone CH<u>H</u>), 4.37 (1 H, m, α CH), 3.85 (1 H, m, alkyl C<u>H</u>OH), 2.09 (4 H, m, alkyl $(CH_2)_2$). **^{13}C NMR** ($CDCl_3$) δ: 171.83 (oxazolidone carbonyl), 152.71 (urethane carbonyl), 135.93, 135.17, 128.38, 128.29, 128.15, 127.93, 127.70, (aromatic C, CH), 77.51 (oxazolidinone CH_2), 67.95, 67.88, 67.80, 67.63, 67.60, (phosphonobenzyl CH_2, benzyloxy CH_2), 61.38, 60.07, (alkyl CH), 54.42, 54.26, (CH), 27.09, 26.88, 26.18, (alkyl CH_2). **IR** (film) υ: 3294 (s), 1799 (s), 1714 (s) cm^{-1}. **Calcd for $C_{28}H_{30}NO_8P$•0.5 H_2O**: C, 61.31; H, 5.70; N, 2.55 **Found**: C, 61.19; H, 5.92; N, 3.08. **Mass spectrum:** decomposes. $[\alpha]_D^{20}$ (c 2.2, $CHCl_3$): -50.

Preparation of 40



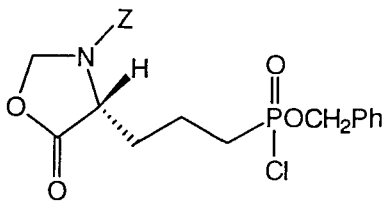
Thiocarbonyldiimidazole (280 mg, 1.41 mmoles) was added to a stirred solution of **39** (500 mg, 0.93 mmole) in 1,2-dichloroethane (13 mL) and the solution was heated, under nitrogen, to 40° C. After 4 h dichloroethane (25 mL) was added and the solution was cooled to 0° C and stirred rapidly with water (8 mL) for 15 min. The organic layer was separated, washed successively with 1 M hydrochloric acid (2 x 10 mL), saturated sodium bicarbonate (15 mL) and sodium chloride (20 mL), dried over magnesium sulfate and evaporated to give **40** as a thick yellow oil (526 mg, 87%). **^{1}H NMR** ($CDCl_3$) δ: 8.17 (1 H, s, imidazole), 7.36 (15 H, m, aromatic), 7.01 (1 H, s, imidazole), 6.00 (1H, m, alkyl $CH_2C\underline{H}$), 5.52 (1 H, d, oxazolidinone $C\underline{H}H$, 5.0 Hz), 4.99-5.14 (7 H, m, benzyloxy CH_2, two phosphonobenzyloxy CH_2, oxazolidinone $CH\underline{H}$), 4.29 (1 H, m, α CH), 2.06 (4 H, m, alkyl $(CH_2)_2$). **^{13}C NMR** ($CDCl_3$) δ: 183.10 (thiocarbonyl), 171.32 (oxazolidone carbonyl), 152.95 (urethane carbonyl), 137.15, 137.06, 135.46, 132.27, 131.02, 128.86, 128.74, 128.32, 128.27, 128.13, 128.10, 118.04, (aromatic C, CH), 77.83 (oxazolidone CH_2), 76.13 (d, J_{PC}=168.22 Hz, alkyl CH (1 diastereomer)), 76.07 (d, J_{PC}=168.02 Hz, alkyl CH (1 diastereomer)), 68.74, 68.68, 68.19, 68.16, (phosphonyl benzyloxy CH_2, benzyloxy CH_2), 54.34, 54.31, (CH), 26.85, 26.75, 24.88 (alkyl CH_2). **IR** (film) υ: 1802 (s), 1720 (s) cm^{-1}. **Calcd for $C_{32}H_{32}N_3O_8PS\bullet 2\ H_2O$**: C, 56.05; H, 5.29; N, 6.13 **Found**: C, 56.08; H, 4.90; N, 6.04. **Mass spectrum:** decomposes. $[\alpha]_D^{20}$ (c 2.2, $CHCl_3$): -45.9.

Preparation of 42



The monochloridate of **41** (122 mg, 0.227 mmole) in dry methylene chloride (0.5 mL) was added over 5 min into a solution of **15** (69 mg, 0.227 mmole) and pyridine (17 μL, 18 mg, 0.227 mmole) in dry methylene chloride (0.6 mL) and stirred at 20° C under dry nitrogen gas for 48 h. The solution was diluted with methylene chloride (3 mL), washed with 0.5 M hydrochloric acid (2 mL), water (2 mL), saturated sodium chloride (2 mL), dried over anhydrous magnesium sulfate and evaporated under reduced pressure to leave 152 mg of a crude oil. Column chromatography (100% ethyl acetate) was employed to isolate **42** as a yellow oil (22 mg, 15%). **^{1}H NMR** ($CDCl_3$) δ: 8.07 (2 H, d, aromatic, 8.3 Hz), 7.33 (15 H, m, aromatic), 7.10 (1 H, s, benzhydryl CH), 7.00 (2 H, d, aromatic, 8.3 Hz), 6.43 (1 H, m, phosphonamidate NH), 5.50 (1 H, m, oxazolidinone C<u>H</u>H), 5.17 (3 H, m, benzyloxy CH_2, oxazolidinone CH<u>H</u>), 4.30 (1 H, m, α CH), 3.70 (3 H, d, phosphonate methyl, 11 Hz), 1.90 (6 H, m, alkyl $(CH_2)_2$).

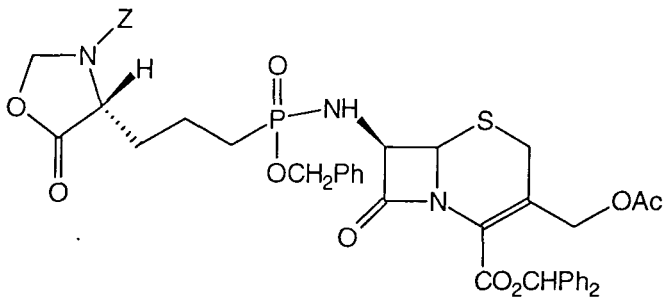
Preparation of 43



Compound **9** (230 mg, 0.44 mmol) was warmed to 48° C and oxalyl chloride (41μL, 60 mg, 0.47 mmole) was added under nitrogen. A sample was removed after 2.5 h and the reaction, according to the NMR

spectrum obtained, was found by to be 50% complete. After 6 h the flask was attached to a vaccuum pump and evacuated at 10 torr for 15 min. The golden residue was dissolved in dry methylene chloride (100 mL) and used immediately to prepare **44**. **^{1}H NMR** ($CDCl_3$) δ: 7.35 (10 H, s, aromatic), 5.51 (1 H, m, oxazolidinone C<u>H</u>H), 5.15 (5 H, m, benzyloxy CH_2, phosphonobenzyloxy CH_2, oxazolidinone CH<u>H</u>), 4.29 (1 H, m, α CH), 1.75 (6H, m, alkyl $(CH_2)_2$).

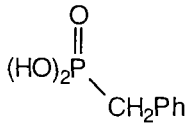
Preparation of 44



A solution of benzhydryl 7-amino cephalosporanate (390 mg, 0.91 mmole) in dry methylene chloride (700 μL) was injected into a solution of **43** (180mg, 0.40 mmole) in dry methylene chloride (100 μL). After 16 h. the reaction mixture was refluxed for 2 h, cooled, diluted to 20 mL, washed successiveley with saturated sodium bicarbonate (20 mL), 1 M hydrochloric acid (20 mL), saturated sodium chloride (20 mL), dried over anhydrous magnesium sulfate and evaporated. The crude product was purified by column chromatography (80% ethyl acetate-hexane), to give **44** (70 mg, 21%) as a pale yellow oil. This product was re-chromatographed to partially resolve the diastereomers. **^{1}H NMR** ($CDCl_3$) δ: 7.35 (20 H, m, aromatic), 6.96 (1 H, s, benzhydryl CH), 5.49 (1 H, s, oxazolidinone C<u>H</u>H), 4.9-5.2 (7 H, m, two phosphonobenzyloxy CH_2, 2 β-lactam CH, thiazine C<u>H</u>H), 4.80 (1 H, d, thiazine CH<u>H</u>, 14 Hz), 4.27 (1 H, m, α CH), 3.54 (1 H, d, acetyl C<u>H</u>H, 18 Hz), 3.38 (1 H, d, acetyl CH<u>H</u>, 18

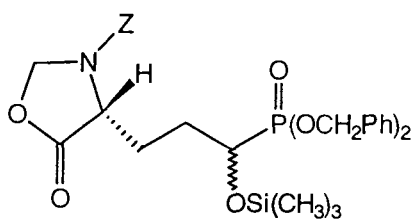
Hz), 2.00 (3 H, acetyl methyl), 1.70 (6 H, m, $(CH_3)_2$). **IR** (film) u: 3400 (w), 1789 (s), 1722 (s), 1230 (s) cm^{-1}.

Preparation of 45



With rapid stirring under nitrogen, trimethylchlorosilane (650 µL, 556 mg, 5.1 mmoles) was injected into a solution of **12** (880 mg, 2.5 mmoles) and sodium iodide (770 mg, 5.1 mmoles) in dry acetonitrile (10 mL). After 1 h the precipitated material was removed by centrifugation and the solution was evaporated to dryness on a rotary evaporator, leaving yellow solids. This material was dissolved in dry toluene (10 mL) and the solution evaporated to give **45** as a hygroscopic powder (290 mg, 60%). **^{1}H NMR** (D_2O) δ: 7.13 (5 H, s, aromatic), 3.00 (2 H, d, benzyl CH_2, 21.0 Hz). **^{13}C NMR** (D_2O) δ: 132.48, 132.42, 131.50, 129.59, (aromatic C, CH) 36.69 (d, J_{PC}=30.2 Hz, benzyl CH_2). **IR** (film) υ: 2784 (s), 1075 (s), 991 (s) cm^{-1}. **Calcd for $C_7H_9O_3P$**: C, 48.85; H, 5.27 **Found**: C, 48.16; H, 5.14.

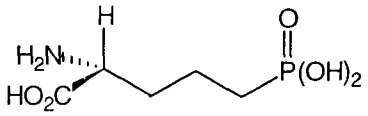
Preparation of 46



Chlorotrimethylsilane (615 µL, 526 mg, 4.84 mmoles) was added to a solution of **11** (846 mg, 3.20 mmoles) and triethylamine (674 µL, 489 mg, 4.84 mmoles) in dry benzene (12 mL) and stirred for 12 h at 20° C. The cloudy mixture was heated to 80° C to drive off excess chlorotrimethylsilane. The solution was cooled to 20° C, transferred via canula to a solution of **38** (1.0 g, 1.79 mmoles) in dry benzene (5 mL) and stirred for 2 h. The mixture was filtered to remove triethylamine

hydrochloride and evaporated to a crude syrup. These products included **46**, which was then hydrolyzed to the alcohol **39** in 95% acetic acid after stirring for 1 h.

Preparation of R-AP5



9 (70 mg, 0.13 mmole) was dissolved in dry ethanol (1 mL) and stirred while palladium on carbon (10%, 150 mg) was added. The flask was placed in a bomb, purged thrice each with nitrogen and hydrogen and then pressurized to 500 psi with hydrogen for 1 h with stirring. The catalyst was subsequently filtered off on a 3 cm pad of Celite and washed with dry ethanol (30 mL) and water (30 mL). The ethanol was removed under reduced pressure and the remaining water was evaporated by lyophilization. The pale white product was suspended in water (5 mL), filtered through a 0.4 μm syringe filter and lyophilized. The crude white residue (15 mg) was purified via HPLC (100% water, 5mL/min, Waters© Bondapak® C18 125A 15-20 μm 25x100 mm column) to yield R-AP5 (5 mg, 20%). **^{1}H NMR** (D_2O) δ: 3.80 (1 H, t, α CH, 6.0 Hz), 1.93 (2 H, m, alkyl PCH_2), 1.59 (4 H, m, alkyl $(CH_2)_2$).

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