IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

UNDERSTANDING SYNTHESIS AND BIOLOGICAL ACTIVITY OF PHOSPHINOPHOSPHONATE PHOSPHOANTIGENS

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Abstract:

The creation of novel phosphoantigens is desirable for a variety of reasons, including prospective pharmaceutics and research into the mechanism of action of existing phosphoantigens. To this date, the phosphoantigens produced by our software have all been phosphonates, which are compounds containing only one phosphorus. All of the synthetic phosphonates, on the other hand, are less powerful than the naturally occurring HMBPP. Bodec and colleagues examined the utility of a second phosphorus by creating a diphosphonic acid, which they used to test their hypothesis.

Keywords: phosphoantigens, diphosphonic acid, phosphinophosphonate,prodrug, prenyl

1. INTRODUCTION

As when the phosphonate phosphoantigen 25b was permitted to activate VΓ9VΔ2 T-cells, a significant increase in potency was seen when the diphosphonic acid 26 was allowed to stimulate VΓ9VΔ2 T-cells, with an EC50 of 0.91 nM vs 2530 nM. When comparing the activity of this diphosphonic acid to that of other phosphonates, it appears that the addition of a second phosphorus group enhances the activity of the compound. The diphosphonate equivalents of DMAPP and IPP showed similar action to the original compounds. HMBPP was more effective than its diphosphonate mimic, but the DMAPP and IPP derivatives were more effective than the naturally occurring diphosphates in the same situations. Even though these compounds are less powerful than the naturally occurring HMBPP, they exhibit an improvement in stability while keeping similar activity characteristics to the natural molecule.

When Hsiao and colleagues investigated the action of phosphoantigen prodrugs, they discovered that it had increased. As a result of this approach, which concealed the charged phosphonate, the BTN3A1 protein was able to diffuse more easily across the cell membrane and attach to the intracellular domain of the protein. If the activity of a prodrug is compared to the activity of the equivalent phosphonic acid, the activity increases 500-fold. When considering diphosphorus compounds, it is possible to imagine analogues of HMBPP and compound 26 as prodrugs that would improve the activity and bioavailability of the medication in question. While it may appear that preserving HMBPP as the oxymethyl pivalate ester would be the best option, this method would result in a highly unstable anhydride. This putative counterpart of HMBPP would be very vulnerable to nucleophilic attack by water, making it an unattractive prodrug candidate for further investigation and development. The similar problem

may arise in the case of a diphosphonate equivalent that has been safeguarded. However, even when stored at low temperatures, it has been shown that comparable prodrug phosphonates, such as compound 60, are water sensitive and decompose within a few days, despite the fact that they are protected methylene bisphosphonate analogues. This breakdown may be observed as a result of a rearrangement involving the lone pair electrons situated on the phosphoryl oxygen in an assault on the isoprene olefin that was similar to an SN2' attack. After hydrolysis of the POM ester, another potential process of breakdown may be observed in a similar way. The phosphinophosphonate substructure was considered to be a preferable strategy for protected analogues due to its ability to bypass the possible stability concerns. Because numerous intermediates of the IBP serve as phosphoantigens, synthesis of the protected counterpart utilizing a phosphinophosphonate skeleton may possibly result in the production of a variety of new T-cell stimulants, which would be of particular interest.

2. SYNTHESIS OF PHOSPHINOPHOSPHONATE PHOSPHOANTIGENS

The development of protected analogues of HMBPP, DMAPP, and IPP, followed by assessment and inquiry into their biological consequences, might potentially shed light on the significance of a second phosphorus moiety in the activation of T-cells, according to the researchers.

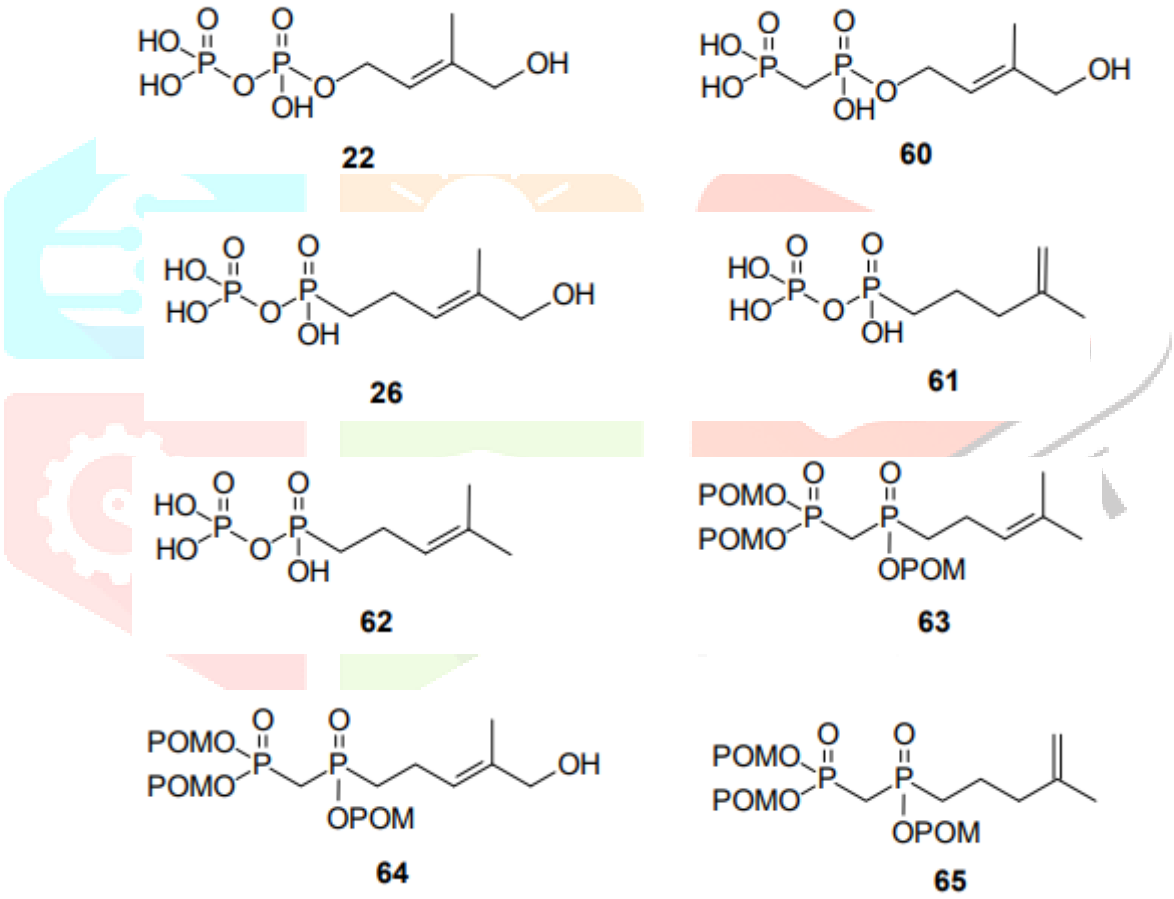

Figure 1. Phosphoantigens containing two phosphorus atoms

A parallel synthesis for the creation of phosphinophosphonates may be accomplished by employing an alkylation technique similar to that used for the formation of dianions from ethyl acetoacetate. In accordance with Savignac's experimental conditions, a nucleophilic addition of the anionic phosphonate 32 to dimethyl methylphosphonate may lead to its condensation, displacement of methanol, and production of the desired phosphinophosphonate 66, followed by the creation of the phosphonate 66. Phosphinophosphonate 66 was synthesised by treating phosphonate 66 with two equivalents of strong base, followed by the addition of prenyl bromide, which resulted in the formation of phosphinophosphonate 66. Savignac and colleagues discovered that dimerization occurred after 5 minutes at 0 degrees Celsius after warming from -78 degrees Celsius. It was discovered that when the identical circumstances were investigated in an attempt to create compound 66, the self-condensation result was not detected and only starting material was obtained.

In order to get the required phosphoantigens, a different approach had to be used because the parent phosphinophosphonate was not identified by this method

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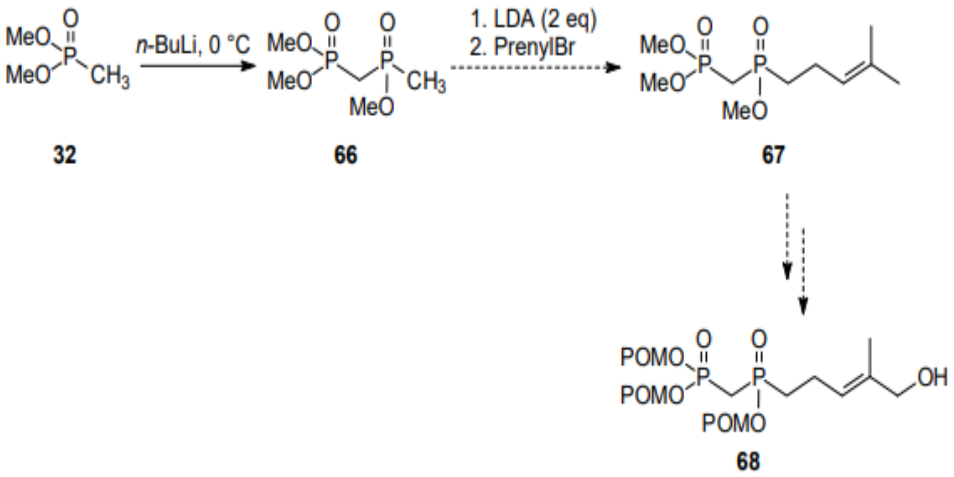

Figure 2. Self-condensation to phosphinophosphonate 66

It is possible that the POM-protected DMAPP and HMBPP phosphinophosphonates will form as a result of a sequence of reactions that occur in parallel. The phosphinophosphonate can be synthesised by reacting the dimethyl homoprenylphosphonate 33 with the equivalent phosphonic acid chloride and the anion of dimethyl methylphosphonate to form the phosphinophosphonate. Using a process similar to that described in the previous chapter, the required chloride could be produced from the appropriate ammonium salt by treating dimethyl homoprenylphosphonate with DABCO after which it was dissolved in water. Dimethyl methylphosphonate was allowed to react with lithium diisopropylamide throughout the actual synthesis, which resulted in the final product (LDA). While investigating the 31P NMR spectra of the reaction mixture, it was discovered that a large number of byproducts had been formed. The creation of a diisopropylphosphonamide might have occurred as a result of the interaction of phosphonic acid chloride 69 with diisopropyl amine, based on the variety of products that were generated. In any case, due to the large number of by-products discovered, an alternate way of obtaining phosphinophosphonate via chloride 69 proved more appealing to researchers.

Recent literature revealed that when dimethyl methylphosphonate was allowed to react with oxalyl chloride and catalytic DMF at room temperature, it could be readily transformed to chloride. Because of its highly reactive nature, the treatment of dimethyl homoprenylphosphonate 33 with oxalyl chloride resulted in the formation of the required phosphonic acid chloride, which was then utilised without additional purification. The reaction between dimethyl methylphosphonate and n-butyl lithium was then allowed to proceed. When chlorophosphonate 69 was added to the anion of dimethyl methylphosphonate after it had formed, it resulted in the production of the required phosphinophosphonate 67 in a moderate yield, which was satisfactory for this application. Using the trimethyl ester 67 as a starting point, allylic oxidation produced the trimethyl ester 70, which is an analogue of the compound HMBPP. The formation of this phosphinophosphonate was verified by examination of the 13C NMR spectra, which revealed that the alpha carbon had been divided by two distinct phosphorus atoms during the process of formation. In this case, the carbon was divided into a pair of pair of pairs with coupling constants of 135.6 Hz and 76.5 Hz respectively.

3. BIOLOGICAL RESULTS

The phosphoantigens portrayed in the section were tested by our partners at the University of Connecticut for their capacity to animate Vγ9Vδ2 T-cells contrasted with the action of known phosphoantigens. We were particularly keen on the action of the phosphinophosphonate as the prodrug dependent on the expanded power saw from the alkyl phosphonate prodrug talked about in Chapter 3. While the alkyl phosphonate prodrug 25c showed a huge increment after concealing the ionizable phosphonic acid, assessment of diphosphonate or diphosphonate ensured analogs has not been

accounted for. Examination of both the prodrugs and phosphonic acids would show the significance of a second phosphorus particle to the action. The aftereffects of the incitement examines are summed up in Table 1 beneath

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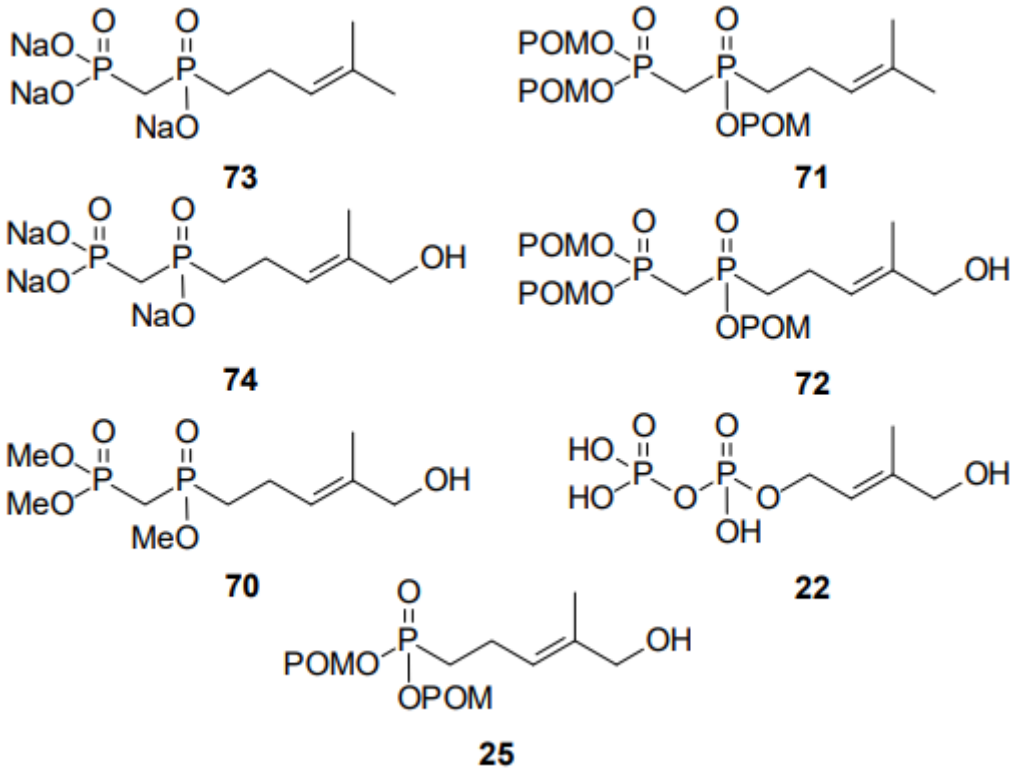

Figure 3. Assayed phosphinophosphonate analogues

Table 1. Vγ9Vδ2 T-cells proliferation and lysis assays

ND = Not Determined

Compound	Expansion ofVγ9Vδ2T-cellsEC$_{50}$(µM)	ToxicitytowardsVγ9Vδ2T-cells IC$_{50}$(µM)	Lysis of K562cells bysimulatedVγ9Vδ2T-cells EC$_{50}$(µM)	Toxicity towards K562 cells IC$_{50}$(µM)
22	0.00051	0.50	0.0016	>100
25c	0.0054	0.60	0.0024	>100
74	26	>100	30	ND
72	0.041	3.5	0.28	3.4
70	>100	>100	>100	ND
7	>100	>100	7.3	9.

1				7
7 3	>100	>100	>100	N D

Our partners tracked down that lone the hydrolysable phosphinophosphonates containing gamma hydroxyl bunches animated development of the Vγ9Vδ2 T-cells (compounds 72 and 74). When the phosphonate ester was not promptly hydrolyzed or there was the absence of allylic liquor, incitement was not seen at focuses under 100 μM. True to form, of the new mixtures the HMBPP simple 72 showed the best incitement of Vγ9Vδ2 T-cells with an EC50 of 0.041 μM. When contrasted with the phosphinophosphonate salt 74, with an EC50 of 26 μM, there is a 600-overlap expansion in incitement when concealing the charged phosphonic acid moiety, like the impacts examined in Chapter 2. Phosphonate 72 showed harmfulness against Vγ9Vδ2 T-cells at a grouping of 3.5 μM with a 85-overlap distinction between incitement versus poisonousness to the cells. This distinction demonstrates a huge remedial window for an expected use as a Vγ9Vδ2 T-cells agonist. With the trimethyl ester 71 at centralizations of up to 100 μM, no recognizable development of Vγ9Vδ2 T-cells was noted. Mixtures 71 and 73, analogs of DMAPP likewise, were examined for their capacity to animate expansion of Vγ9Vδ2 T-cells without an allylic liquor. In the two cases, these mixtures were idle up to 100 μM fixations, reaffirming the significance of the gamma hydroxyl usefulness on the capacity of potential phosphoantigens to invigorate Vγ9Vδ2 T-cells development.

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4. SYNTHESIS OF IPP PHOSPHINOPHOSPHONATE ANALOGUE

While these previous analogs permitted clarification of some key unthinking attributes identified with phosphoantigens and Vγ9Vδ2 T-cells incitement by our associates, union of an IPP subsidiary would be another significant objective. With the limiting information that was resolved, the IPP simple could have a more grounded restricting liking than the normally happening agonist. Combination of this IPP simple and examination of its action to the regular IPP likewise could highlight the significance of boost the SAR by adding another phosphorous Using Iodide 76, the IPP phosphinophosphonate may be ready to use in a matter of minutes. In this way, IPP phosphinophosphonate was combined in a similar way to what had been described before. Three-methyl-3-butenone was allowed to react with triphenylphosphine, imidazole and iodine in order to reduce the cost of the optimal essential iodide 76 with triphenylphosphine oxide as a by-product. A mixture of the ideal item and the phosphine oxide was used in section chromatography to seclude the 76 iodide. Iodide refining attempts to clean the iodide came to fruition just as degradation was occurring. An expected polymerization or hydrogen iodide disposal to frame isoprene could cause this breakdown. When liquor 75 is treated with TsCl, followed by NaI, a non-essential iodide can be obtained. Again, attempts to remove the iodide proved dangerous because of its low stability and rapid disintegration

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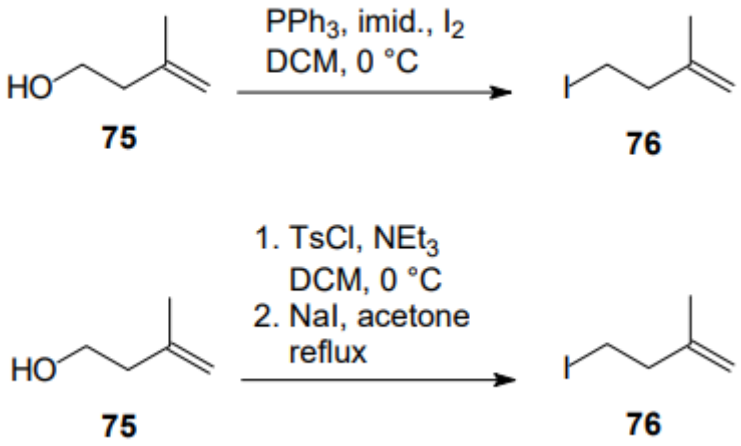

Figure 4. Attempts at formation of the primary iodide

Recent work has indicated that the production of the main bromide 77 may be accomplished using MsCl, followed by a Finkelstein reaction with LiBr in situations where the iodide 76 decomposes rapidly. This technique resulted in the isolation of the main bromide 77 in 51 percent of the cases. Using the same approach that Zgani used to produce the diphosphonate 26, the monophosphonate 78 would be accessible following the formation of the dimethyl methylphosphonate anion, according to the proposed scheme. The phosphonate anion was treated with the bromide 77, which resulted in the formation of the phosphonates 78 and 33, which were mixed in a one-to-two ratio. After 4 hours, the process had not reached its completion point, and a significant amount of starting material remained in solution.

It is possible that the production of phosphonate 33 is owing to an isomerization reaction mediated by a base, which results in the creation of the more stable alkene. With the addition of base to bromide 77, it is possible that base isomerization will occur, resulting in the formation of the more stable trisubstituted olefin through an allylic anion. By sharing comparable pka values with the trisubstituted olefin and increasing its concentration, the allylic anion has the potential to promote the production of the olefin and decrease the quantity of terminal alkene present in the reaction mixture. Attempts to avoid this isomerization were made by varying the time of the reaction and the temperature of the reaction. When the reaction durations were reduced, both isomers were found in low yields and 1:1 ratios, indicating that the reaction was incomplete. When the reaction time was prolonged, the ratios shifted in favour of the production of the trisubstituted olefins. Furthermore, when using column chromatography, it was not possible to distinguish between the terminal and trisubstituted olefins. As a result of the difficulty encountered in separating combinations of these isomeric olefins and the poor yielding stages required, a more selective synthesis strategy was required to generate the possible phosphoantigen 85.

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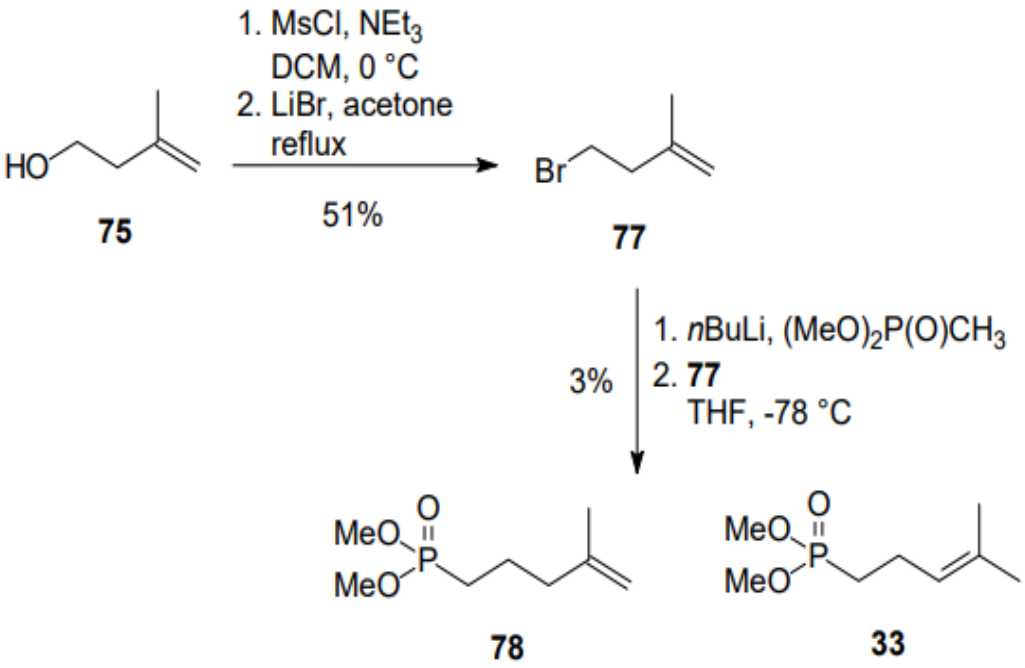

Figure 5. Unexpected rearranged olefin

The 1, 4-addition of dimethyl methylphosphonate anion to methyl vinyl ketone was the focus of the second method utilised to achieve the synthesis of phosphinophosphonate 85 in the laboratory. But there was no sign of the intended product, and the ^{31}P NMR spectrum revealed a single resonance that corresponded to the starting material. There was yet another method under consideration in order to avoid the problems that had arisen from the prior paths. In the alternate method, a Wittig reaction was utilised to instal the terminal olefin from levulinic acid, which was successful. Methylenetriphenylphosphonium bromide was used to make the carboxylic acid 80, which was produced after it was treated with n-BuLi at 0 °C. Levulinic acid was then added to any excess ylide to produce the carboxylic acid 80. The isolation of the resultant acid was made simple by using column chromatography in conjunction with acetic acid to maintain a neutral species during the process.

5. CONCLUSION

As a result of the treatment with MsCl and triethyl amine, the primary alcohol 81 was converted into the mesylate 82 with almost quantitative yields. Using the mesylate as a starting point, a MichaelisBecker reaction might occur by reaction with dimethyl hydrogen phosphonate, followed by treatment with the base. Under previously described circumstances, the dimethyl hydrogen phosphonate was allowed to react with NaH before being combined with the mesylate to create the phosphonate 78, which was obtained in poor yield. It was necessary to optimize the process once again in order to increase the production of the required phosphonate. It was discovered that the solubility of the developing anion in THF reduced substantially as a result of the addition of base. Once it was determined that the solubility was poor, the investigation of additives and alternate bases to improve solubility began.

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