NEW APPLICATIONS FOR BIOCATALYSIS IN ORGANIC SYNTHESIS

By

G. NEIL STOWE

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2008

ACKNOWLEDGMENTS

I would like to thank Dr. Stewart for his steadfast guidance throughout my graduate school career. I would like to thank Dr. Brent Feske for teaching me how to function in a bio-organic lab when I started working. Thanks go to (the soon to be Dr.) Jordan Mathias for listening to my unique thoughts on the joys of research and for being one of the best friends I have ever had. Thanks go to Despina Bougioukou for being a better source of useFULL information than an Encyclopedia. Thanks go to Dimitri Dascier for teaching me how to behave like a true Parisian. Thanks go to Adam Rothman for informing me of the true price of a pack of cigarettes. Thanks go to Paige Finkelstein -the kiddo- for being my constant source of entertainment for 10 weeks and showing me how to less than three chemistry.

Thanks go to Richard Farley, Josh Bunger, Jenna Norton, Ashlee Mantooth, Lisa Nackers and Janet Cusido and the other members of MUSCO for giving me something fun and competitive to do outside of the lab. Thanks go to Jonathan Sommer for teaching me how to surf and showing me how to stop working and have a good time. Thanks go to Lisa Nackers for smiling and laughing. Heartily.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

NEW APPLICATIONS FOR BIOCATALYSIS IN ORGANIC SYNTHESIS

By

G. Neil Stowe

December 2008

Chair: Jon D. Stewart
Major: Chemistry

We synthesized a series of alpha-methyl-beta-ketoesters in two steps. These ketoesters were then reduced to the corresponding alpha-methyl-beta-hydroxyesters by our library of purified ketoreductases from Baker's Yeast and Baker's Yeast whole cells. These steps allowed us to probe the reduction capabilities and specificities of our purified ketoreductases and also demonstrated that they yield far greater enantioselectivities in comparison to whole cells.

A phosphonic acid analog of the highly toxic chemical warfare agents Sarin, Soman and VX was synthesized in four steps. This analog was tested as a target against DNA aptamers as a part of a program to develop a rapid test for exposure to these nerve agents in the field. Unfortunately, no aptamers bound to the target.

We chemoenzymatically synthesized alpha-fluoro-phosphonic acid carbohydrates in seven steps from noncarbohydrate precursors. The stereochemistry of the alpha-fluorine was controlled by selective reduction of an alpha-fluorovinylphosphonate using our library of alkene reductases. The alpha-fluorinated phosphonate alkane reduction product could then be used with aldolase and a suitable acceptor aldehyde to make five and six membered alpha-fluorinated phosphonic acid carbohydrates of defined stereochemistry without the need for protection / deprotection steps.

CHAPTER 1
ENZYMATIC ROUTE FOR THE SYNTHESIS OF CHIRAL BUILDING BLOCKS FROM ALPHA-METHYL-BETA-KETOESTERS

Introduction

Selective reductions of β-ketoesters can result in valuable chiral building blocks for organic synthesis. These reductions, which control the stereochemistry of an alpha substituent in addition to the β-alcohol, can be accomplished using Baker's Yeast whole cells that can be purchased at any supermarket (Figure 1-1).[1] However, multiple reductases with potentially different reduction products are present in whole yeast cells; this can significantly decrease the optical purity of the reduced product.[2-5]

In 2004, Kaluzna et al.[5] made a library of 18 purified β-ketoreductases from Baker's yeast which were expressed as fusion proteins with glutathione *S*-transferase (GST) to allow for simple purification. The library of purified reductases was screened against a variety of ketoester substrates to yield chiral α-substituted-β-hydroxyesters (Figure 1-2).[5]

These reductases were overexpressed in *E. coli* whole cells, allowing for gram scale, selective reductions of α-chloro-β-ketoesters without the need for expensive cofactor regeneration systems. The resulting chiral α-chloro-β-hydroxyesters were then used to efficiently synthesize both antipodes of the Taxol side chain **9a-b**[6] and (-)-Bestatin **10**[7] (Figure 1-3).

We wanted to further determine the influence of the substituent at the α-position by comparing electronic versus steric environments while also expanding our knowledge of substrates for our enzyme library. So, α-methyl-β-ketoesters **11a-d** were synthesized and screened (Figure 1-4); these β-ketoesters are similar in size but different electronically to the previously screened α-chloro-β-ketoesters **6a-d**.[8]

Previous methods for asymmetric synthesis of α-methyl-β-hydroxyesters employed biocatalytic reduction of α-methyl-β-ketoesters[9] in addition to ring opening of optically pure epoxides with Grignard reagents[10] and aldol reactions with chiral oxazoborolidinone reagents[11] (Figure 1-5). These approaches were limited: the biocatalytic method offered a small quantity of reductases which gave a limited number of chiral products while chemical methods required chiral starting materials or reagents. We thought that our library of reductases could prove superior to these methods because we had previously shown with α-chloroesters **6a-d** that multiple chiral products could be made from racemic starting materials.

Thus, if the α-chlorine of the previously screened α-chloro-β-ketoesters was seen by our enzymes as only a steric entity, then we hypothesized that substrate acceptance and product stereochemistry should be similar for the α-chloro and α-methyl compounds. However, if our enzymes could discern between the different electronic environments of the sterically similar α-substituents, then we thought that substrate acceptance and product stereochemistry could differ significantly. We also wanted to compare stereochemistry of the alcohol products of nonsubstituted β-ketoesters **3a-b** against the α-methyl-β-ketoesters. Finally, α-methyl-β-ketoesters **11a-d** were tested for reduction with Baker's yeast whole cells to determine if our purified reductases yield better reduction product selectivity in addition to comparison of whole cell substrate acceptance.

Ketoester Synthesis

The α-methyl-β-ketoesters were made using a two step process. First, ethyl propionate was deprotonated by lithium diisopropylamide (LDA) at -78^{o} C followed by addition of the appropriate aldehyde at the same temperature to yield alcohols **12a-d** and **13a-d**.[12] The alcohols were oxidized to ketoesters **11a-d** via standard Swern conditions or PCC (Figure 1-6).[12,13]

Screening

Theα-methyl-β-ketoesters **11a-d** were screened against our library of purified reductases and whole yeast cells. Screenings with purified reductases were conducted in phosphate (KP_i) buffer and used NADPH cofactor as the hydride source. The cofactor was regenerated using a glucose-6-phosphate (G6P) / glucose-6-phosphate dehyrogenase (G6PDH) couple (Figure 1-7).[5] Whole cell screening of ketoesters **11a-d** incubated Fleischman's® yeast with shaking in KP_i buffer with addition of sucrose as needed.

Gas chromatography (GC) was used to monitor reaction progress and selectivity. Aliquots of the crude reaction mixture were monitored by achiral GC in order to follow reaction progress. The stereoselectivity of each reaction was determined by chiral GC using trifluoroacetylated derivatives **19a-d** and **20a-d** of the possible alcohol products **12a-d** and **13a-d** (Figures 1-8, 1-9).

Results and Discussion

The screenings found that only ketoesters **11a** (R = Et) and **11b** (R = *n*-Pr) were substrates for our purified reductases. Both ketoesters were reduced by enzymes encoded by yeast genes YDR541c, YGL039w, YAL060w and YGL157w. Chiral GC analysis of the trifluoroacetylated derivatives found only one of four possible alcohol products was formed from reduction of both ketoesters. NMR analysis of the α-methoxy-α-phenylacetic (MPA) esters of the alcohol showed both reductions yielded α-methyl-β-hydroxy esters with 2(*S*), 3(*S*) configurations, corresponding to *anti*-**13a** and *anti*-**13b**. Whole yeast cells also accepted only **11a** and **11b** as substrates, but gave a 50:50 enantiomeric mixture of *anti*-**12a-b** or *anti*-**13a-b** as the major products. (Table 1-1).

The α-chloro-β-ketoesters **6a-d** proved far better substrates for our purified enzymes than the α-methyl compounds; both **11a** (R = Et, α-methyl) and **11b** (R = *n*-Pr, α-methyl) were accepted by four purified enzymes while **6a** (R = Et, α-chloro) and **6b** (R = *n*-Pr, α-chloro) were

accepted by 14 and 7 purified enzymes, respectively. However, the major product stereochemistry with respect to the alcohol remained the same for unsubstituted, α-chloro and α-methyl-β-ketoesters. Thus, the hydride was delivered predominately to the same face of the ketone. The α-substituent of the major product was also in the same position for both α-chloro and α-methyl-β-ketoesters, showing that our purified enzymes preferred to accept the same enantiomer of starting material. (Figure 1-10, Table 1-1, Table 1-2).

From a synthetic perspective, our results from reduction of α-methyl-β-ketoesters **11a-b** with purified enzymes were successful since we could produce an optically pure product from racemic starting material. However, our method would prove more versatile if more than one chiral product could be made.

The generalizations made with respect to reduction of unsubstituted, α-chloro and α-methyl-β-ketoesters with our purified enzymes could be made with whole yeast cells in some cases. For example, α-methyl **11a** (R = Et) gave a 50 : 50 ratio of *anti* enantiomeric products in 92% de which was similar to the 38 : 50 *anti* enantiomeric ratio obtained for α-chloro **6a** (R = Et). Whole cells were more diastereoselective for reduction of α-methyl **11b** (R = Pr) than α-chloro **6b**, but **6b** still gave a 53 : 36 enantiomeric mixture of *anti* alcohols as major products.

However, these generalizations did not always hold true. For example, reduction of unsubstituted β-ketoesters **3a** and **3b** was selective, especially for **3b**, but the hydride was added from the opposite face of the ketone during whole cell reduction when the alpha substituent was not present.

The results of purified enzyme versus whole cell reductions were not surprising since multiple reductases are present in whole cells which often give different products. In fact, during this project we discovered from whole cell screening of α-methyl-β-ketoesters **11a-b** that

additional reductases, which could lead to additional optically pure products, are present in the yeast genome. These reductases can be isolated and purified via GST fusion protein methodology in the future. However, our current library has proven an excellent method for the production of valuable chiral products from readily or commercially available, racemic starting materials.

Experimental Procedures

Materials

All organic chemicals, G6P and G6PDH were purchased from Sigma-Aldrich and used without further purification. Diisopropylamine and triethylamine (NEt_3) were freshly distilled from CaH_2 before use. Dimethylsulfoxide (DSMO) was dried overnight over molecular sieves and then freshly distilled before use. *n*-Butyllithium concentration was determined by titration with 2,5-dimethoxybenzyl alcohol. THF, ether and CH_2Cl_2 were degassed in 20 L drums and passed through two sequential purification columns (activated alumina) under a positive argon using the GlassContour system (GlassContour, Inc.). Thin Layer Chromatography (TLC) was performed on Merck TLC glass sheets with visualization by UV light or staining using potassium permanganate or vanillin. ^1H (300) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Mercury 300 spectrometer. Chemical shifts (δ) for ^1H and ^{13}C NMR are given in parts per million (ppm) relative to TMS and referenced relative to residual protonated solvent ($CHCl_3$: δ_H 7.27 ppm, δ_C 77.00 ppm or C_6D_6: δ_H 7.16 ppm, δ_C 128.39). Some carbon signals were isochronous for racemic alcohols and α-methyl-β-ketoesters. Compounds were separated via GC with an HP 5890 Series II Gas Chromatograph equipped with an achiral DB-17 column and Chirasil-Dex CB or Chirasil Beta-Dex columns (0.25 mm x 25 m x 0.25 μm thickness). GC / MS used an HP 5890 Series II Gas Chromatograph equipped with an achiral DB-17 column (0.25 mm x 25 m x 0.25 μm thickness).

GC short runs were 60° C for 2 minutes, 10° C per minute ramp to 180° C followed by 10 minutes at 180° C. GC long runs were 60° C for 2 minutes, 1° C per minute ramp to 150° C, 10° C per minute ramp to 180° C followed by 10 minutes at 180° C. GC / MS runs were 60° C for two minutes, 10° C per minute ramp to 250° C followed by 10 minutes at 250° C.

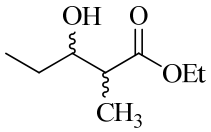
Synthesis

Titration of *n*-Butyllithium.

An oven dried round bottom flask was charged with 2,5-dimethoxybenzylalcohol (0.225 g / 1.34 mmol) and 8 mL of dry THF. *n*-Butyllithium (583 μL) was then added dropwise at room temperature until a dark brown color persisted. At this point, just over 1 equivalent of *n*-Butyllithium had been added, and the concentration of the solution was determined to be 2.3 M.

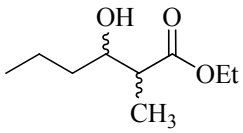
General Procedure for synthesis of alcohols **7a-d** and **8a-d**.

A round bottom flask was flame dried while purging with Argon, cooled, and charged with dry tetrahydrofuran (THF) (20 mL) and diisopropylamine (3.1 mL, 22.1 mmol), then cooled to -78° C. *n*-Butyllithium (*n*-BuLi) (20.1 mL, 22.1 mmol) was then added dropwise at -78° C and the resulting solution stirred at the same temperature for 30 minutes. Ethyl propionate (2.1 mL, 20.3 mmol) in THF (5 mL) was then added dropwise to the solution of LDA at -78° C and the resulting solution stirred at the same temperature for one hour. The appropriate aldehyde (22.4 mmol) in THF (5 mL) was then added dropwise at -78° C and the resulting solution stirred at -78° C for 30 minutes before the addition of 8 mL of saturated ammonium chloride. After warming to room temperature the mixture was poured into 150 mL of ether and washed with 10 mL of saturated sodium chloride (NaCl). The organic layer was dried with magnesium sulfate ($MgSO_4$) and the solvent removed under reduced pressure to give the crude product which was purified by flash chromatography.



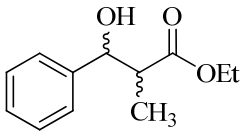
syn-**12,13a** + *anti*-**12,13a**

Purified by flash chromatography (6:1 Hexanes : EtOAc) to give the pure compound as a slightly yellow oil in 75% yield as a 55 : 45 mixture of diastereomers.[10] ^{1}H NMR ($CDCl_3$): δ 0.97 (m, 6H). 1.17 (d, 3H, *J* = 7.1 Hz), 1.15 (d, 3H, *J* = 7.4 Hz), 1.27 (t, 6H, *J* = 7.1 Hz), 1.54 (m, 4H), 2.52 (m, 2H), 2.58 (br d, 2H *J* = 7.0 Hz), 3.58 (m, 1H), 3.81 (m, 1H), 4.17 (q, 4H, *J* = 7.1 Hz). ^{13}C NMR ($CDCl_3$): δ 10.01, 10.65, 10.89, 14.44, 14.68, 26.97, 27.83, 44.12, 45.05, 60.77, 60.80, 73.41, 74.92, 176.35, 176.58. FT-IR: (neat) 3500, 2954, 1728, 1182. MS: 145 (M-15, 0.5%), 131 (47.0%), 115 (25.6%), 102 (89%), 85 (66%) 74 (100%), 57 (48.3%).



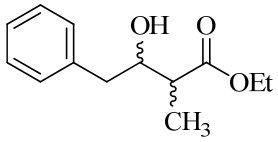
syn-**12**,**13b** + *anti*-**12**,**13b**

Purified by flash chromatography (8:1 Hexanes : EtOAc) to give the pure compound as a slightly yellow oil in 70% yield as a 53 : 47 mixture of diastereomers.[11] ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 6H *J* = 7.4 Hz), 1.17 (d, 3H, *J* = 7.4 Hz), 1.23 (d, 3H, *J* = 7.1 Hz), 1.27 (t, 6H, *J* = 7.1 Hz), 1.45 (m, 8H), 2.45 (m, 2H), 2.55 (br d, *J* = 7.0 Hz), 3.59 (m, 1H), 3.90 (m, 1H), 4.17 (q, 4H, *J* = 7.1 Hz). ^{13}C NMR ($CDCl_3$): δ 10.97, 14.20, 14.31, 14.49, 14.62, 18.95, 19.86, 36.22, 37.10, 44.59, 45.56, 60.71, 60.82, 71.64, 73.30, 176.49, 176.76. FT-IR: (neat) 3500, 2960, 1732, 1187. MS: 174 (m/z, 0.2%), 159 (1.9%), 131 (26.2%), 102 (100%), 74 (90.5%), 57 (31.8%).



syn-**12,13c** + *anti*-**12,13c**

Purified by flash chromatography (10:1 Hexanes : EtOAc) to give the pure compound as a slightly yellow oil in 60% yield as a 50 : 50 mixture of diastereomers.[14] ^{1}H NMR ($CDCl_3$): δ 1.01 (d, 3H, *J* = 7.1 Hz), 1.14(d, 3H, *J* = 7.1 Hz), 1.21 (t, 3H, *J* = 7.2 Hz), 1.25 (t, 3H, *J* = 7.2 Hz), 2.75 (m, 2H), 3.05 (br s, 2H), 4.13 (q, 2H, *J* = 7.1 Hz), 4.19 (q, 2H, *J* = 7.1 Hz), 4.65 (m, 1H), 5.05 (m, 1H), 7.35 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 11.08, 14.21, 14.29, 14.63, 46.63, 47.32, 60.87, 60.93, 73.89, 76.49, 126.19, 126.83, 127.61, 128.15, 128.36, 128.60, 141.66, 141.78. FT-IR: (neat) 3450, 3063, 1716. MS: 208 (m/z, 5.3%), 193 (0.6%), 163 (3.7%), 133 (24.7%), 102 (100%), 74 (70.0%), 57 (17.5%).



syn-**12,13d** + *anti*-**12,13d**

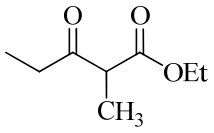
Purified by flash chromatography (10:1 Hexanes : EtOAc) to give the pure compound as a slightly yellow oil in 50% yield as a 51 : 49 mixture of diastereomers. ^{1}H NMR ($CDCl_3$): δ 1.27 (m, 12H), 2.65 (m, 4H), 2.79 (m, 4H), 3.90 (m, 1H), 4.18 (m, 5H), 7.25 m, (10H). ^{13}C NMR ($CDCl_3$): δ 11.20, 14.40, 14.45, 14.62, 40.60, 41.48, 43.82, 44.54, 60.83, 60.93, 72.93, 74.68, 126.79, 128.77, 128.81, 129.53, 129.67, 138.32, 138.40, 176.24. FT-IR: (neat) 3490, 2938, 1731. MS: 204 (M – 18, 31.2%), 159 (14.9%), 131 (100%), 85 (72.6%), 57 (32.0%).

General Procedure for the synthesis of ketones **11a-d**.

Swern Oxidation: An oven dried round bottom flask was charged with dimethylsulfoxide (DMSO) (1.27 mL, 17.9 mmol) in CH_2Cl_2 (20 mL) and cooled to -60^o C before the dropwise addition of oxalyl chloride (1.42 mL, 16.5 mmol) in CH_2Cl_2 (5 mL) at the same temperature. After complete addition, the solution was stirred at -60^o C for 10 minutes before dropwise addition of alcohol (14.3 mmol) in 5 mL of CH_2Cl_2 at -60^o C. The solution was stirred at -60^o C

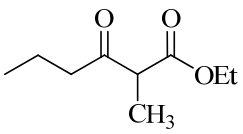
for 20 minutes after complete alcohol addition and then quenched by dropwise addition of triethylamine (NEt_3) at the same temperature. The resulting solution was allowed to warm to room temperature over a one hour period before the removal of CH_2Cl_2 under reduced pressure. The resulting residue was dissolved in ether (25 mL), washed with H_2O (5 mL) and brine (5 mL) and the organic layer dried with $MgSO_4$. The solvent was removed under reduced pressure to give the crude ketone which was purified by flash chromatography.

Pyridinium chlorochromate (PCC) oxidation: A round bottom flask was charged with 50 mL of anhydrous CH_2Cl_2 and PCC (3.93 g, 18.2 mmol). The alcohol (9.1 mmol) in 5 mL CH_2Cl_2 was then added dropwise to the solution (The color of the solution rapidly changed from bright orange to brown). The solution was then stirred for four hours at room temperature before the addition of 50 mL of anhydrous ether. The supernatant was then separated from the black precipitate and the precipitate was washed with three portions of ether (3 x 10 mL). The solvent was removed under reduced pressure, and the product purified by flash chromatography.



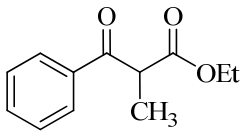
11a

Prepared by Swern oxidation in 70% yield as a colorless oil after flash chromatography (8 : 1 Hexanes : EtOAc).[12] ^{1}H NMR ($CDCl_3$): δ 1.08 (t, 3H J = 7.3 Hz) 1.26 (t, 3H, J = 7.2 Hz), 1.36 (d, 3H, J = 7.2 Hz), 2.58 (m, 2H), 3.57 (q, 1H J = 7.2 Hz), 4.25 (q, 2H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$): δ 12.3, 14.1, 35.6, 51.0, 61.3, 169.9, 206.5. FT-IR: (neat) 2970, 1741, 1715. MS: 158 (m/z, 3.6%), 129 (7.9%), 113 (11.7%), 102 (37.8%), 74 (28.3%), 57 (100%).



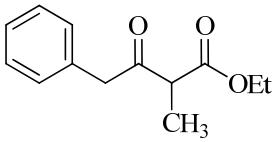
11b

Prepared by Swern oxidation in 61% yield as a colorless oil after flash chromatography (8 : 1 Hexanes : EtOAc).[15] ^{1}H NMR ($CDCl_3$): δ 0.91 (t, 3H, *J* = 7.3 Hz), 1.27 (t, 3H *J* = 7.2 Hz), 1.34 (d, 3H, *J* = 7.2 Hz), 1.61 (m, 2H), 2.55 (m, 2H), 3.50 (q, 1H *J* = 7.2 Hz) 4.20 (q, 2H, *J* = 7.0 Hz). ^{13}C NMR ($CDCl_3$): δ 12.6, 13.9, 16.8, 43.1, 52.6, 52.8, 61.2, 170.5, 205.8. FT-IR: (neat) 2965, 1743, 1716. MS: 172 (m/z, 3.4%), 143 (1.3%), 127 (8.8%), 102 (32.0%), 74 (100%), 57 (12.5%).



11c

Prepared by PCC oxidation in 72% yield as a colorless oi after flash chromatography (12 : 1 Hexanes : EtOAc).[16] ^{1}H NMR ($CDCl_3$): δ 1.18 (t, 3H *J* = 7.2 Hz) 1.50 (d, 3H *J* = 7.0 Hz), 4.16 (q, 2H *J* = 7.2 Hz), 4.40 (q, 1H, *J* = 7.2 Hz), 7.45 (m, 2H), 7.59 (m, 1H), 8.0 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 13.9, 14.2, 48.6, 61.6, 128.8, 128.9, 133.7, 136.1, 171.1, 196.2. FT-IR: (neat) 2985, 1744, 1684. MS: 206 (m/z, 9.9%), 161 (2.5%), 133 (0.8%), 102 (100%), 77 (36.4%).

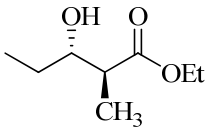


11d

Prepared by PCC oxidation in 50% yield as a colorless oil after flash chromatography. [17] ^{1}H NMR ($CDCl_3$): δ 1.26 (t, 3H *J* = 7.2 Hz) 1.32 (d, 3H *J* = 7.2 Hz), 3.62 (q, 1H *J* = 7.2 Hz), 3.82 (s, 2H), 4.19 (q, 2H *J* = 7.2 Hz), 7.25 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 12.9, 14.2, 48.7, 52.0, 61.5, 127.7, 128.8, 129.7, 133.6, 170.5, 203.4. FT-IR: (neat) 2985, 1730, 1700. MS: 220 (m/z, 19.9%), 174 (6.9%), 129 (44.3%), 102 (16.1%), 91 (100%), 74 (10.4%).

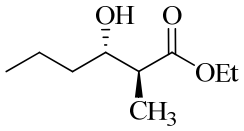
General procedure for the synthesis of optically pure alcohols *anti*-**8a** and *anti*-**8b**:

A round bottom flask was charged with $NADP^+$ (7 mg), glucose-6-phosphate (86 mg), Glucose-6-phosphate dehydrogenase (50 μg), α-methyl-β-ketoester (95 μL) and purified reductase (2.2 mL) in 6 mL of KP_i buffer. The flask was then incubated with gentle shaking at 30 ^oC for 36 hours. The reaction mixture was then extracted with ether (3 x 15 mL), the combined organics were dried with $MgSO_4$, and the solvent removed under reduced pressure to give the crude product as an oil.



anti-**13a**

Purification by flash chromatography (12 : 1 Hexanes : EtOAc) gave the pure product as a slightly yellow oil in 80% yield.[18,19] ^1H NMR ($CDCl_3$): δ 0.96 (t, 3H J = 7.4 Hz), 1.20 (d, 3H, J = 7.1 Hz), 1.26 (t, 3H J = 7.1 Hz), 1.51 (m, 2H), 2.50 (dq, 1H J_1 = 7.0 Hz, J_2 = 7.0 Hz), 2.65 (br d, 1H, J = 7.0 Hz), 3.58 (m, 1H), 4.17 (q, 2H J = 7.1 Hz). ^{13}C NMR ($CDCl_3$): δ 10.02, 14.38, 14.53, 27.77, 45.00, 60.73, 74.85, 176.30. FT-IR: (neat) 3500, 2969, 1730, 1185. $[\alpha]_D$ = +3^o (C = 0.3, $CHCl_3$). MS was the same as **12-13a**.



anti-**13b**

Purification by flash chromatography (12 : 1 Hexanes : EtOAc) gave the pure product as a slightly yellow oil in 80% yield.[11] ^1H NMR ($CDCl_3$): δ 0.90 (t, 3H J = 7.1 Hz), 1.20 (d, 3H, J = 7.1 Hz), 1.24 (t, 3H, J = 7.1 Hz), 1.50 (m, 4H), 2.45 (dq, 1H, J_1 = 7.0 Hz, J_2 = 7.0 Hz), 2.59 (br d, 1H, J = 7.0 Hz), 3.59 (m, 1H), 4.15 (q, 2H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$): δ 14.17, 14.37, 14.44, 18.93, 37.05, 45.48, 60.71, 73.25, 176.31. FT-IR: (neat) 3500, 2964, 1733, 1186. $[\alpha]_D$ = +4^o (C = 0.2, $CHCl_3$). MS was the same as **12-13b**.

General Procedure for MPA alcohol derivitization:

An NMR tube was charged with one equivalent of alcohol, $CDCl_3$ (1 mL), and one equivalent of a mixture of (*R)*-MPA and (*S*)-MPA (2 : 1), followed by addition of 1.5 equivalents of dicyclohexylcarbodiimide (DCC) and 0.5 equivalents of N,N-dimethylaminopyridine (DMAP).[8] The crude reaction was monitored by ^1H and 1D TOCSY NMR. The chemical shift differences, listed as the change in chemical shift between (*R*) and (*S*) esters (□δ*R*,*S*), are summarized in Table 3.

The chemical shift differences between (*R*) and (*S*) MPA esters allowed us to assign (*S*) absolute stereochemistry for both alcohols.[20,21] We determined relative configuration for both compounds based on vicinal coupling constants ($^3J_{HH}$) for the H_2 signal (7.0–7.5 for *anti* and 2.5-3.0 for *syn*)[22] and comparison of the H_3 chemical shift for *anti* and *syn* isomers with literature data. For these β-hydroxyketones (R = Me, Et, *i*-Pr, Ph, Bn), H_3 is shifted upfield for *anti* isomers.[11,14,19,23]

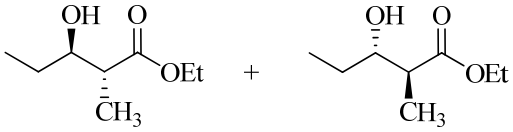
For α-methyl-β-hydroxyester **13a** (R = Et), $^3J_{HH}$ of 7.0 Hz for H_2 in addition to an H_3 chemical shift identical with literature data (δ 3.58 observed, lit. value: δ 3.58 (*anti*), 3.84 (*syn*))[19] allowed us to conclude *anti* relative stereochemistry, assigning the full configuration as 2(*S*), 3(*S*).

For α-methyl-β-hydroxyester **13b** (R = *n*-Pr), $^3J_{HH}$ of 7.0 Hz for H_2 in addition to an upfield chemical shift for H_3 (δ 3.59 from purified enzyme reduction vs. 3.59 and 3.90 from nonselective aldol reaction) allowed us to conclude *anti* relative stereochemistry, assigning the full configuration as 2(*S*), 3(*S*). This assignment does not agree with Hena et al., who published chemical shifts of H_3 for *anti* and *syn* isomers as δ 3.7 and 3.5, respectively.[11] However, Hena et al. did not report any coupling constants for H_2 to support their assignment. Thus, we believe the

configuration was not assigned correctly since other α-methyl-β-hydroxyesters in this series (R = Me, Et, *i*-Pr, Ph, Bn) assigned the chemical shift of H_3 for *anti* compounds upfield from *syn*. And, our coupling constants support *anti* relative stereochemistry, while Hena et al. did not report this data.

General procedure for reductions of α-methyl-β-ketoesters using Baker's Yeast whole cells:

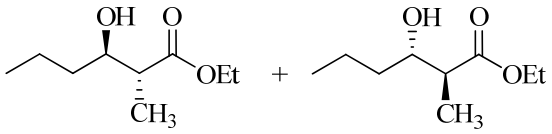
Fleishman's® yeast (10 g) was added to a solution of 45 mL of tap water in a baffled Erlenmeyer flask followed by the addition of 15 g of sucrose. The flask was then shaken at 30° C for one hour followed by the addition of 100 μL of substrate. The reaction was then shaken at 30° C for 5 days with addition of sucrose as needed. Aliquots of the reaction mixture were analyzed via chiral and achiral GC. After five days, the reaction mixture was filtered over Celite and the aqueous layer extracted with EtOAc (3 x 75 mL). The combined organics were washed with brine (1 x 8 mL), dried with $MgSO_4$ and the solvent removed under reduced pressure to give the crude compound as a yellow oil which was purified by filtration through a short plug of silica to give the pure compound. For each compound, the major products using a long run were one of two possible peaks on achiral GC and two of four possible peaks on chiral GC.



anti-**12a** *anti*-**13a**

Purified by a short flash chromatography column to give a slightly yellow oil in 60% yield.[24] Alcohols were analyzed as trifluoroacetylated derivatives *anti*-**19-20a** and *syn*-**19-20a**. The products from $NaBH_4$ reduction were separated using an achiral DB-17 column (peaks at 11.14 and 12.50 min, long run) and a chiral Dex column (peaks at 26.39, 27.66, 28.23 and 29.08 min, long run). Trifluoroacetylated derivatives of the reaction products showed peaks at 11.14

and 12.50 min (long run) (1 : 21.5, 91% de, long run) on achiral GC and 27.70, 28.38 and 29.11 min (11.0 : 1.0 : 11.0, 0% ee, long run) on chiral GC. The major products are the *anti* enantiomers since the peak at 27.70 min corresponds to the product obtained from purified enzyme reduction. Our results also agree with the literature.



anti-**12b** *anti*-**13b**

For this compound, alcohols *anti*-**12-13b** and *syn*-**12-13b** were not derivatized. The products from $NaBH_4$ reduction were separated using an achiral DB-17 column (peaks at 22.51 and 24.14 min, long run) and a chiral Dex column (peaks at 53.56, 53.91, 54.58 and 54.90 min, long run). After reaction, product appeared on achiral GC at 22.41 min (>99% de, long run) and on chiral GC at 53.66 and 54.32 (1.0 : 1.0, 0% ee, long run). These are the *anti* enantiomers since the peak at 53.66 min corresponds to the alcohol from purified enzyme reduction.

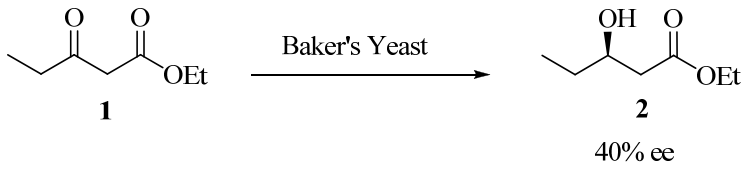


Figure 1-1. Selective reduction using Baker's yeast whole cells

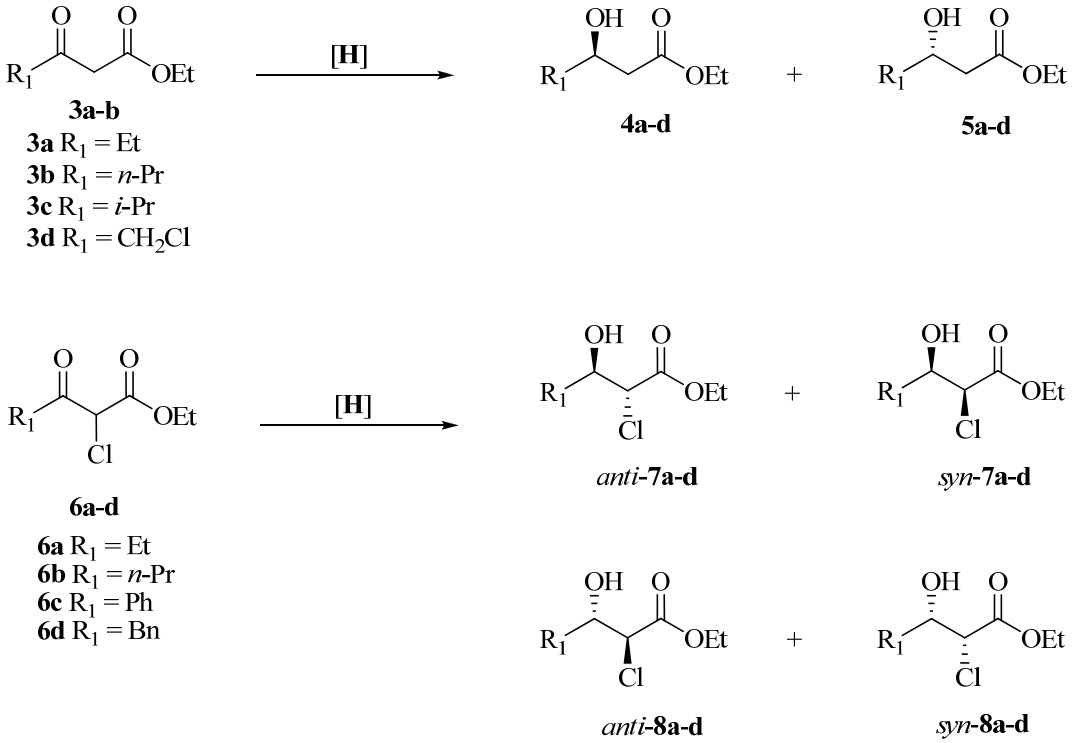


Figure 1-2. Substrates screened by Kaluzna et al.

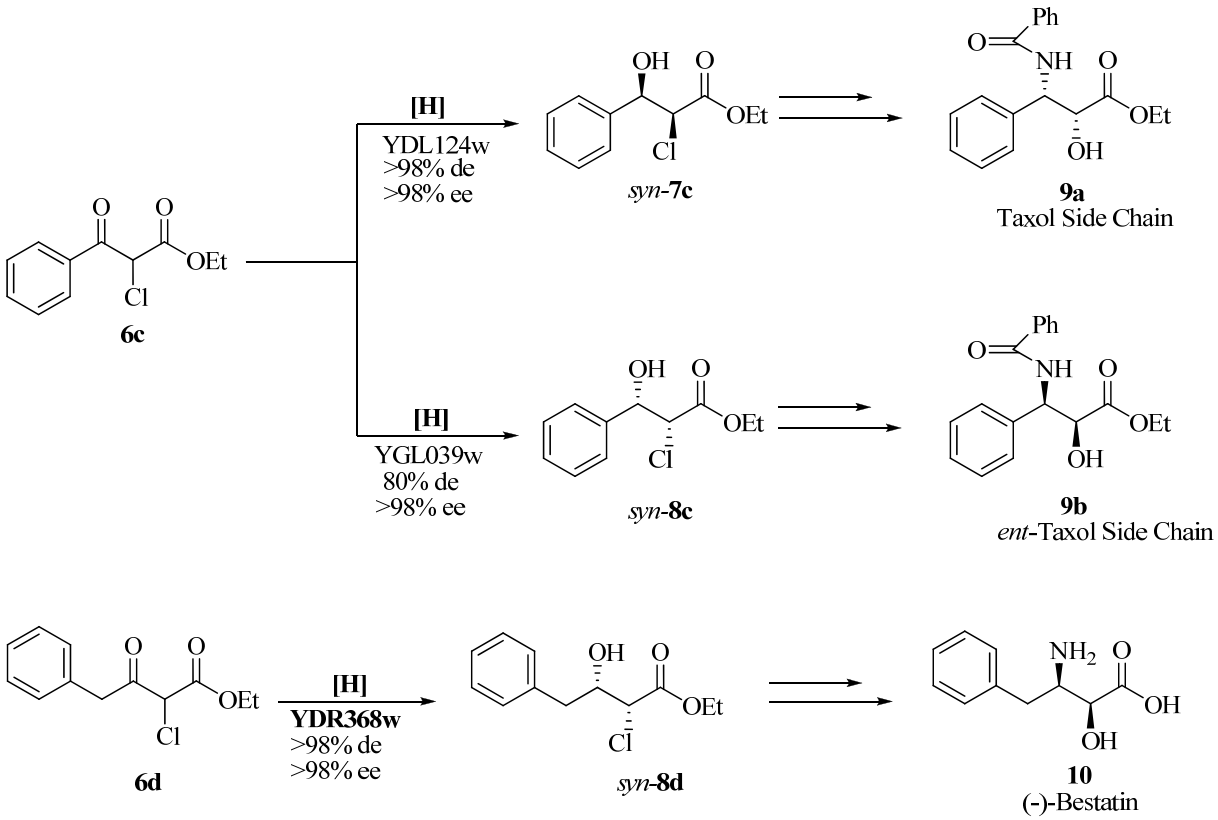


Figure 1-3. Synthetic applications of chiral products from α-chloro-β-ketoester reductions

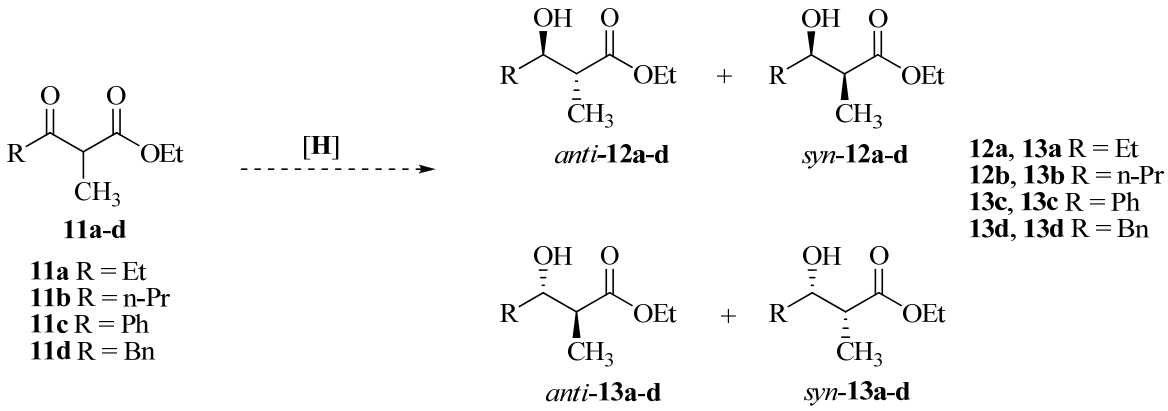


Figure 1-4. The α-methyl-β-ketoesters synthesized and possible alcohol products

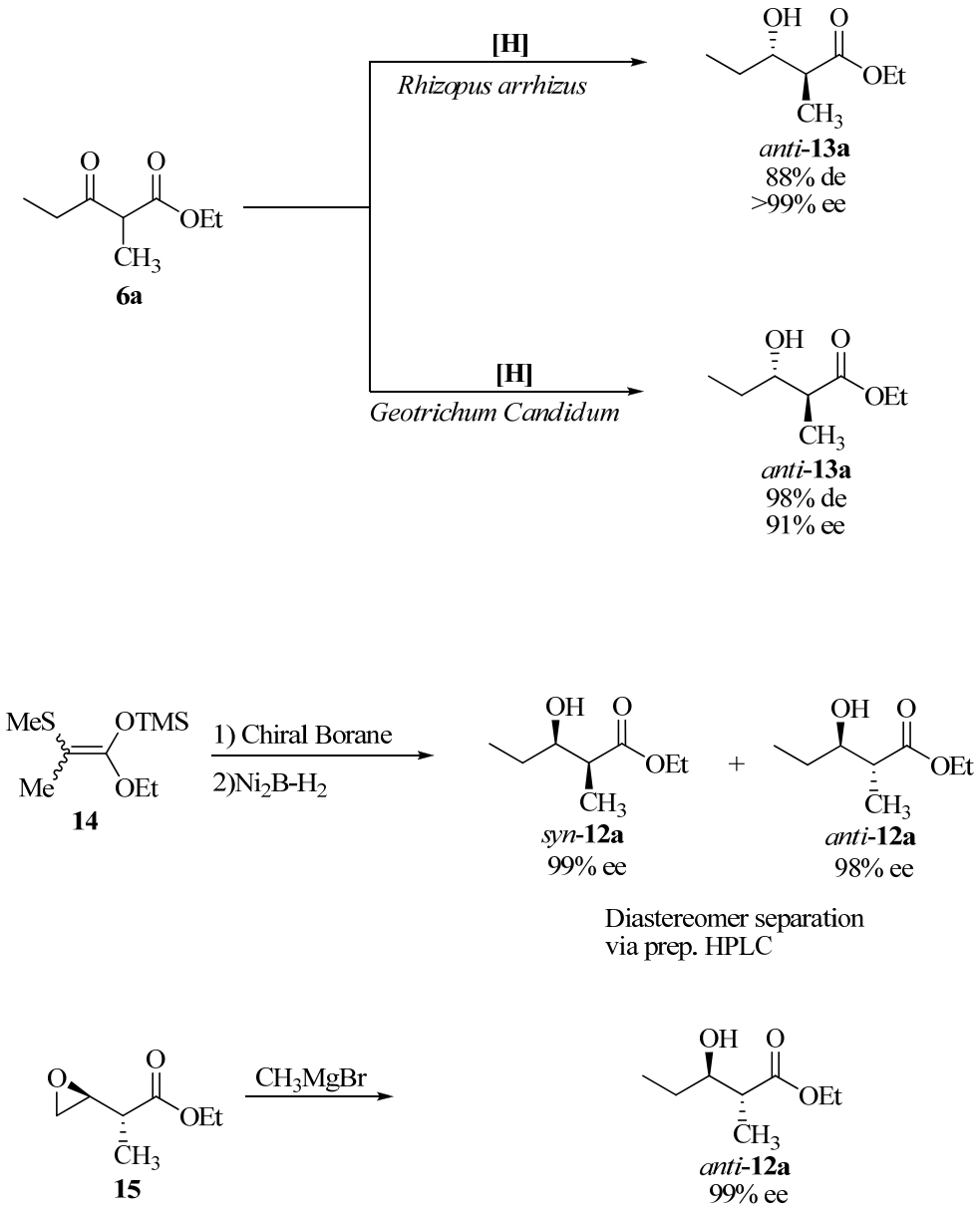


Figure 1-5. Previous methods for selective reduction of α-methyl-β-ketoesters

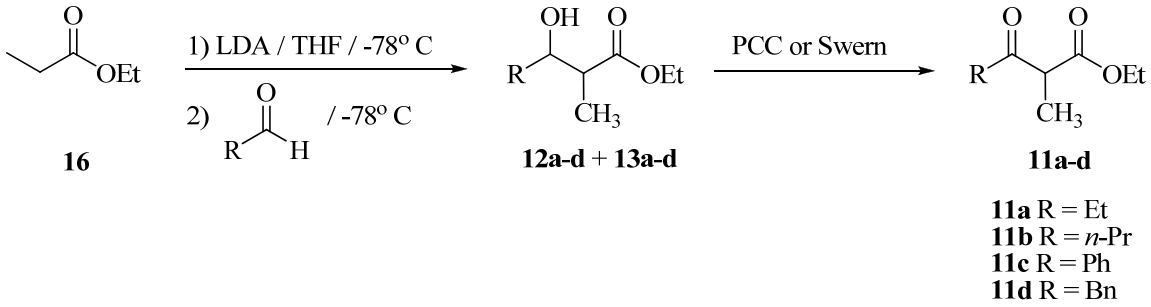


Figure 1-6. Synthesis of α-methyl-β-ketoesters

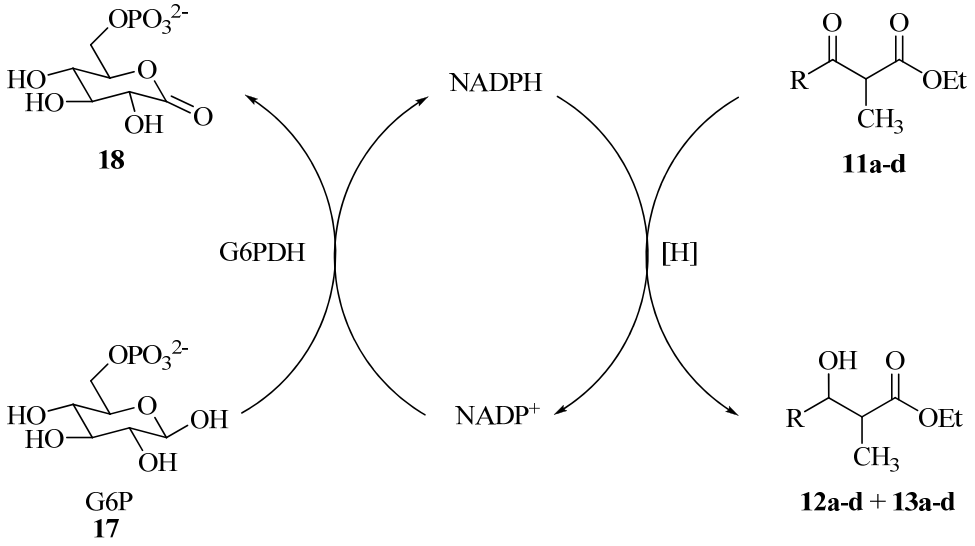


Figure 1-7. Cofactor regeneration with G6PDH

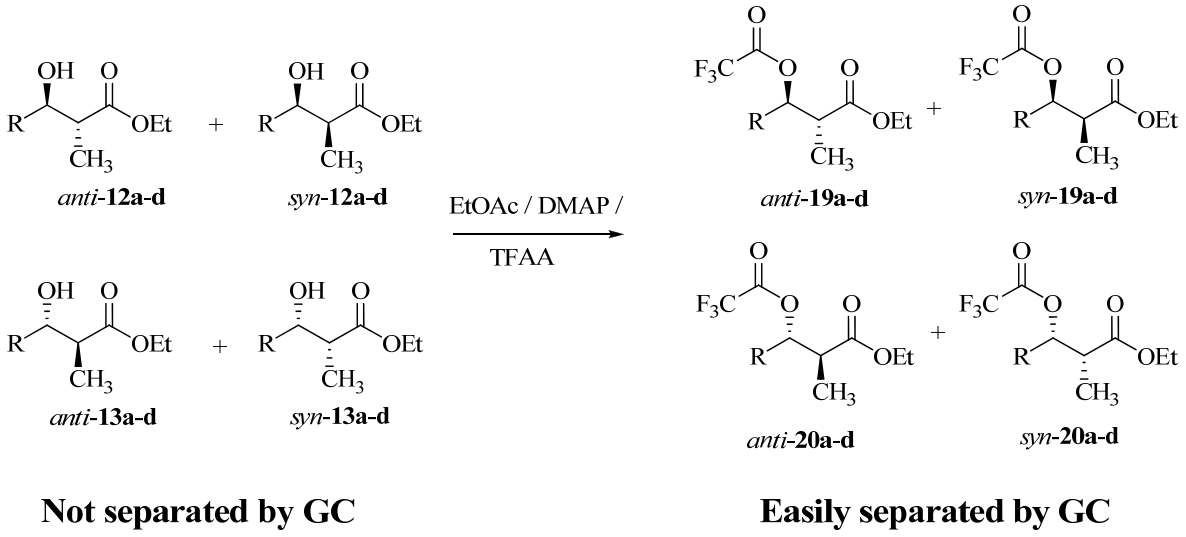


Figure 1-8. Trifluoroacetyl alcohol derivatives

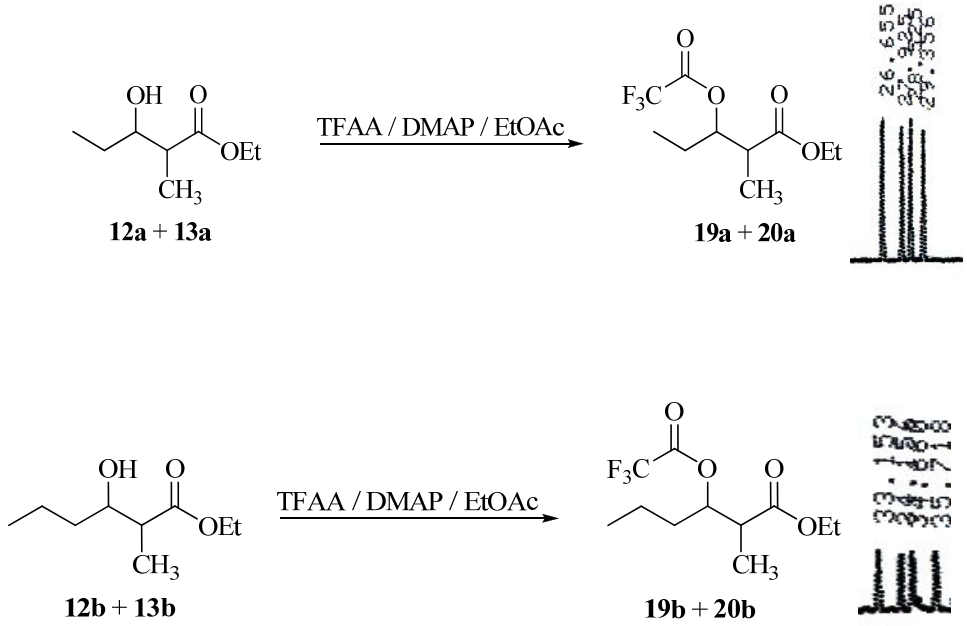


Figure 1-9. Trifluoroacetylation GC values for racemic alcohols

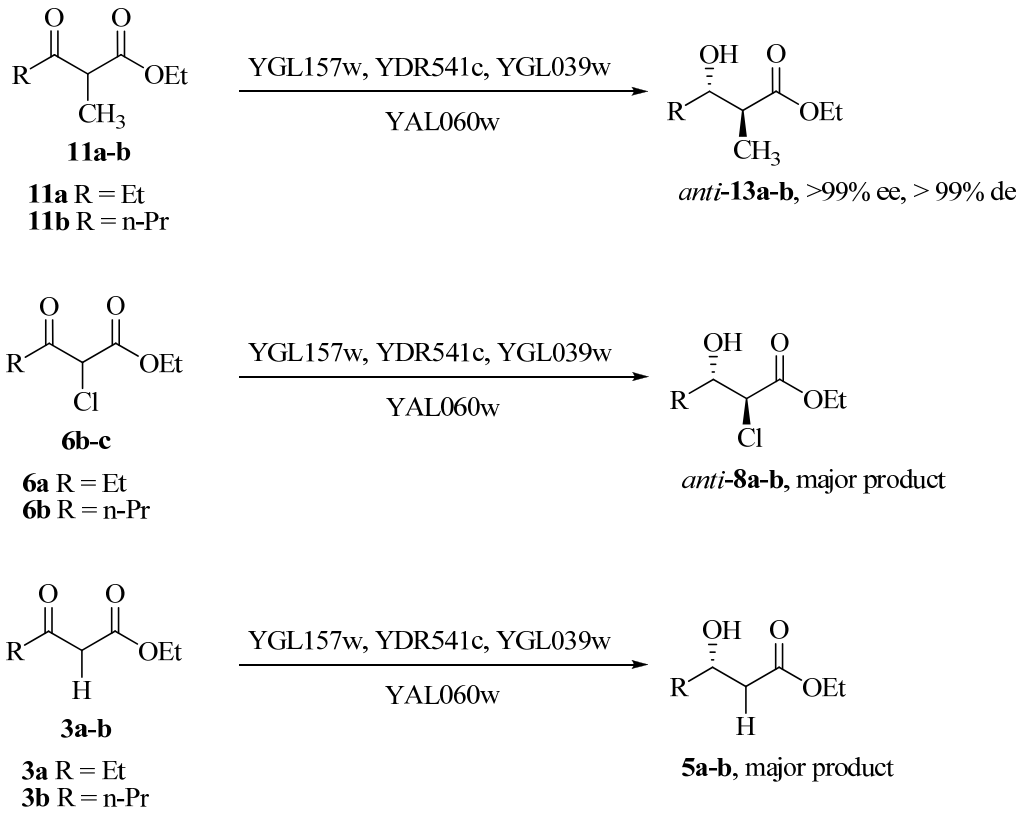


Figure 1-10. Reductions of ketoesters to alcohols with purified enzymes

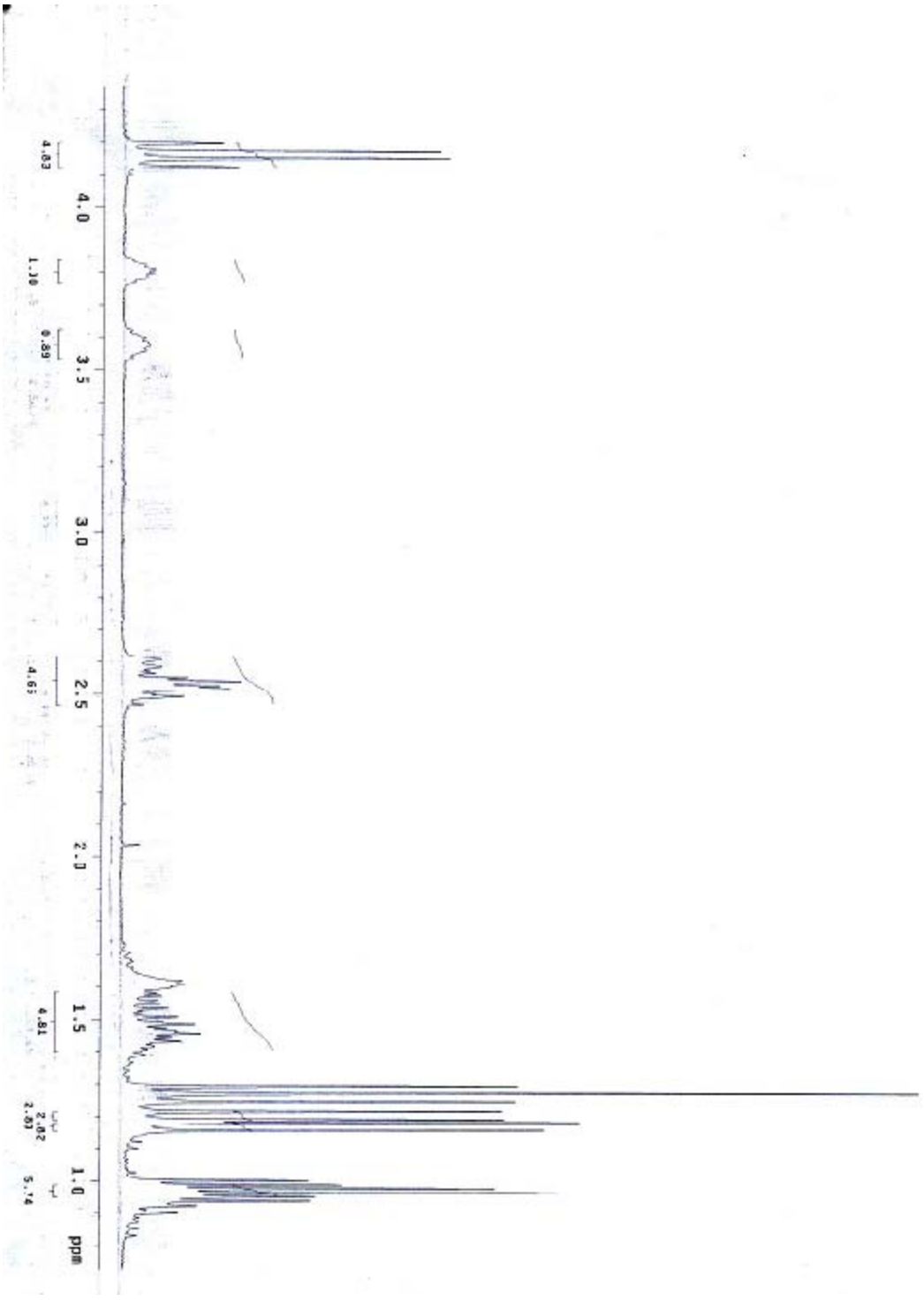


Figure 1-11. *Anti*-**12**,**13a** + *syn*-**12**,**13a**

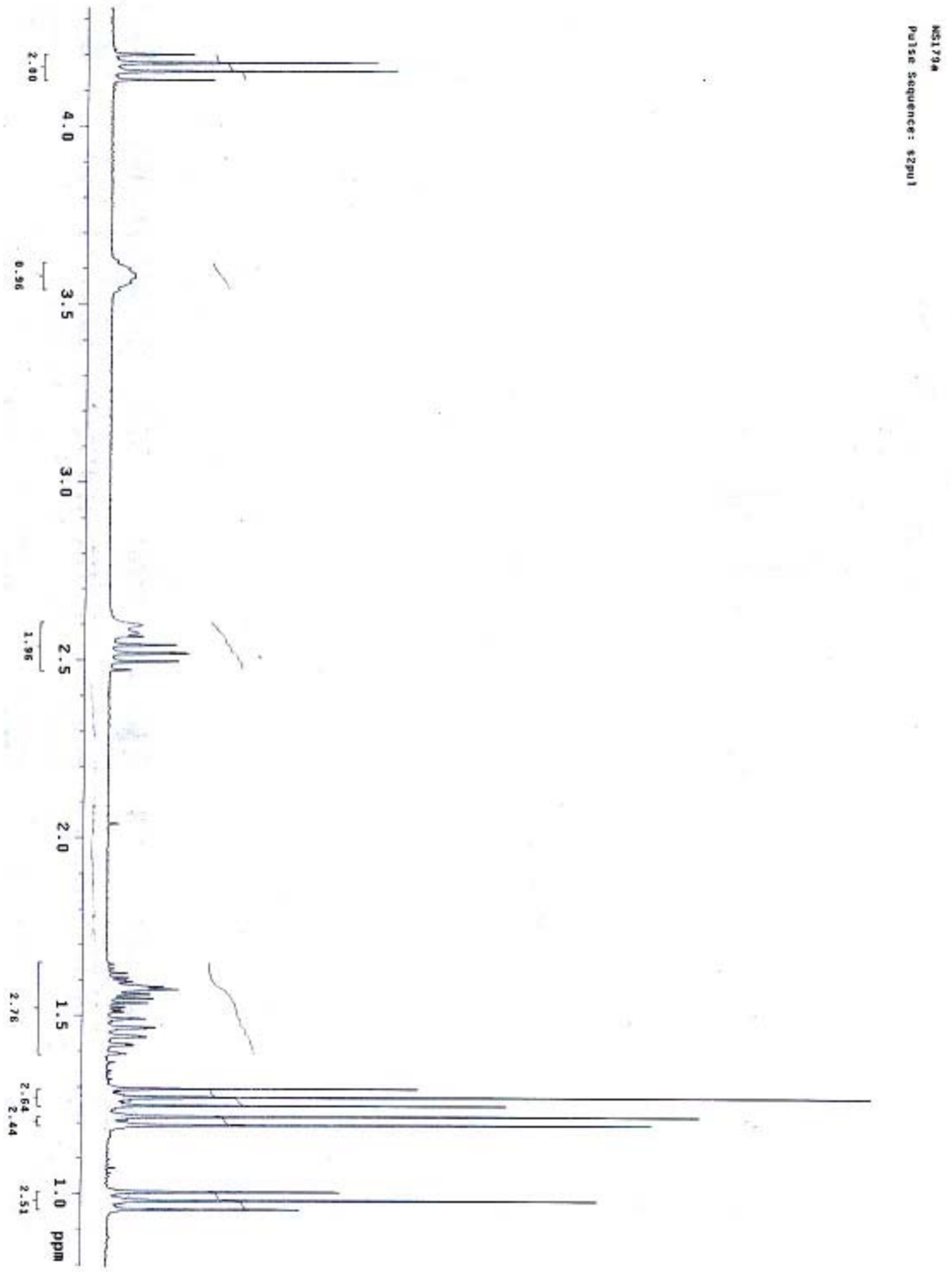


Figure 1-12. *Anti*-**13a**, from enzymatic reduction of ketone **11a**

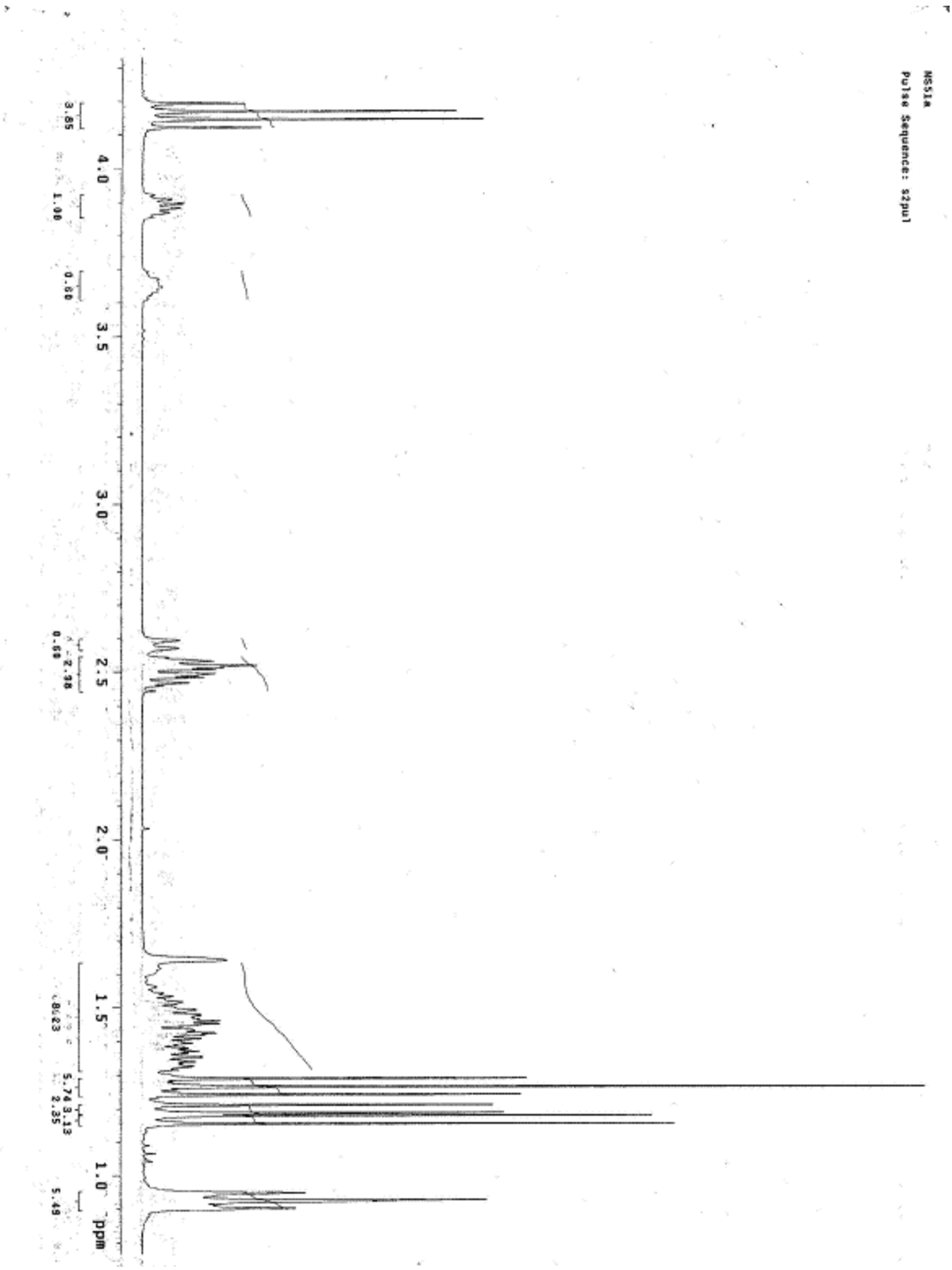


Figure 1-13. *Anti*-**12-13b** + *syn*-**12-13b**

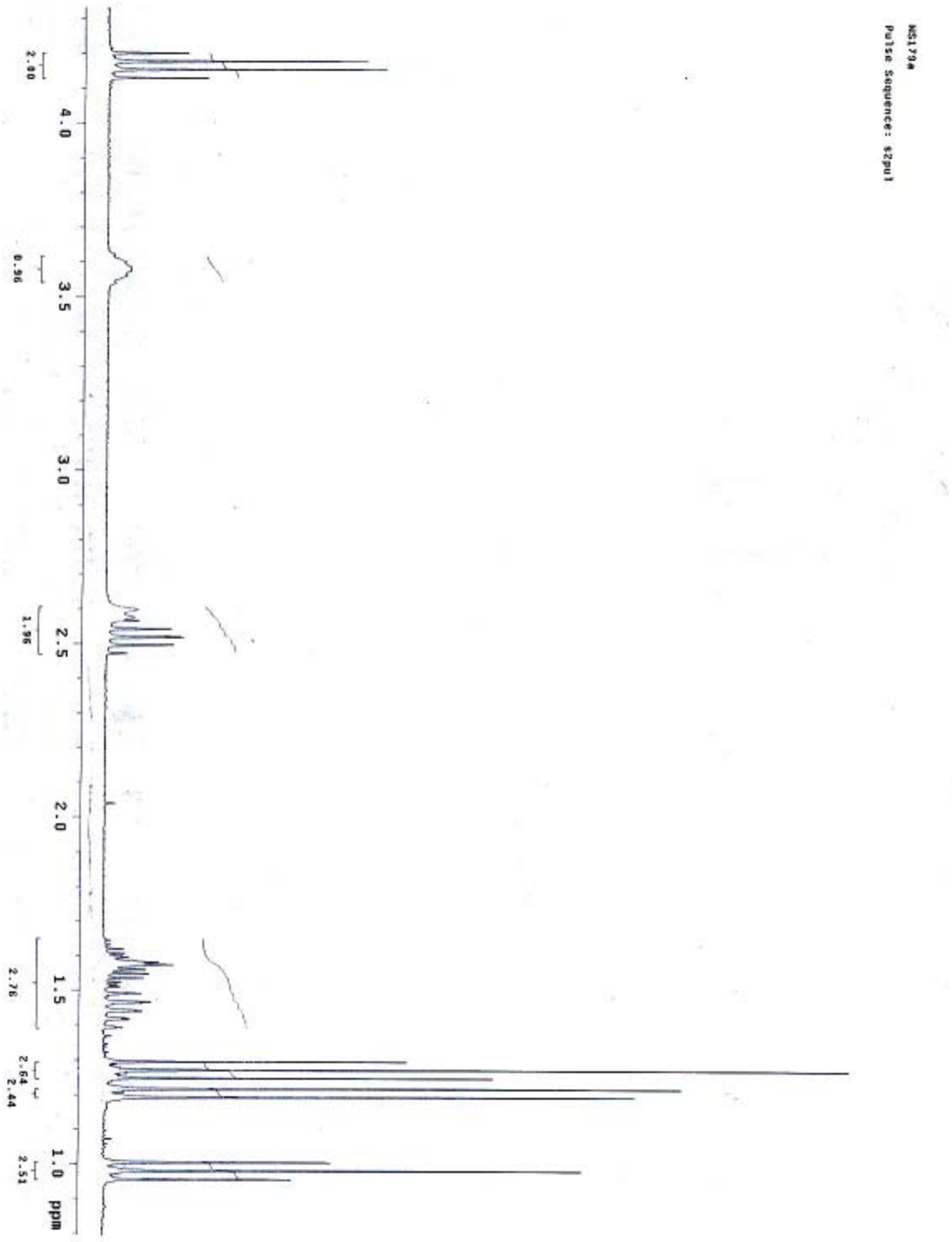


Figure 1-14. *Anti*-**13b** from enzymatic reduction of ketone **11b**

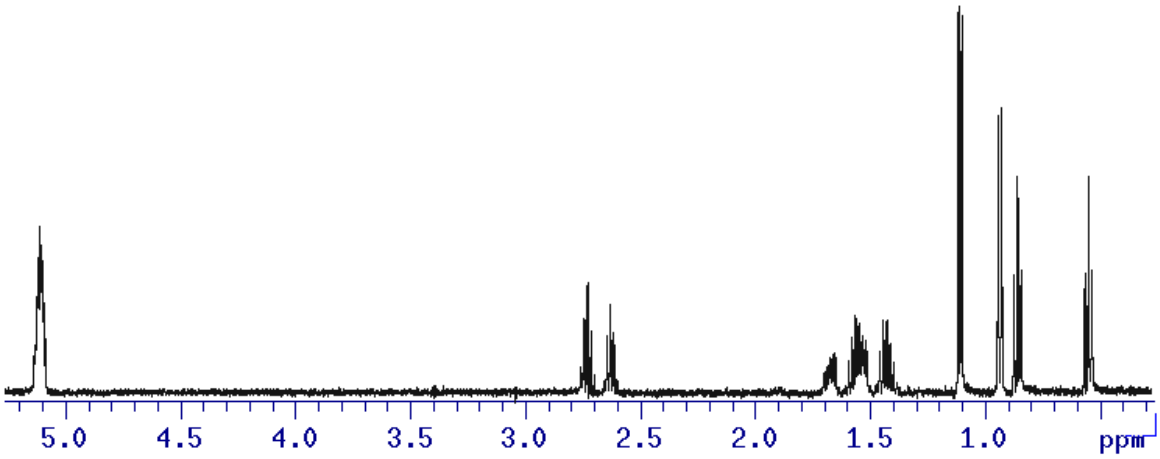


Figure 1-15. 1-D TOCSY NMR of crude (*R*) and (*S*) MPA esters of *anti*-**13a**

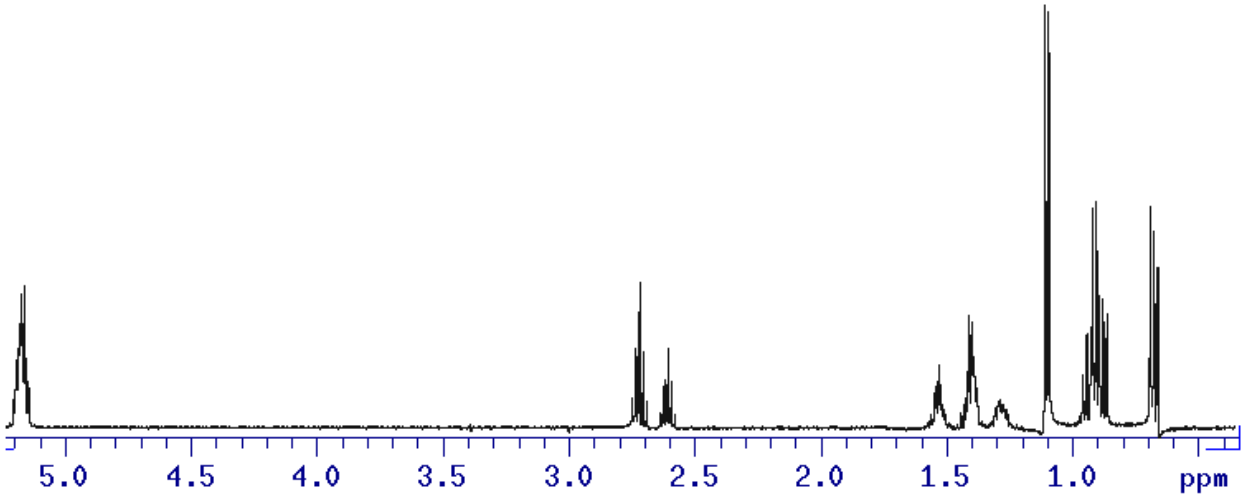


Figure 1-16. 1-D TOCSY NMR of crude (*R*) and (*S*) MPA esters of *anti*-**13b**

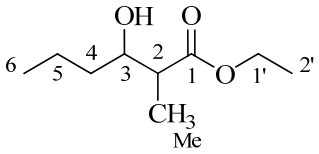
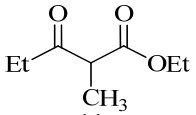
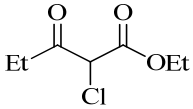
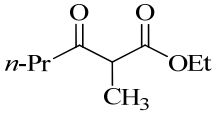
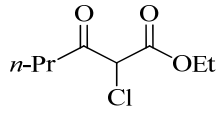


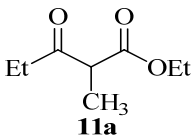
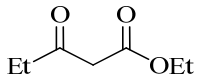
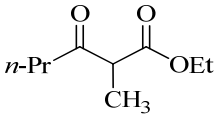
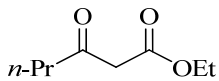
Figure 1-17. Nomenclature used for assignment of configuration

Table 1-1. Comparison of α-methyl and α-chloro-β-ketoester reductions

Yeast Gene	Plasmid	 **11a**	 **6a**	 **11b**	 **6b**
		anti-**12a** : *syn*-**12a** : *anti*-**13a** : *syn*-**13a**	*anti*-**7a** : *syn*-**7a** : *anti*-**8a** : *syn*-**8a** :	*anti*-**12b** : *syn*-**12b** : *anti*-**13b** : *syn*-**13b**	*anti*-**7b** : *syn*-**7b** : *anti*-**8b** : *syn*-**8b** :
YJR096w	pIK9	---	---	---	---
YDL124w	pIK8	---	<1 : 19 : 7 : 73	---	---
YBR149w	pIK12	---	<1 : >99 : <1 : <1	---	---
YOR120w	pIK30	---	<1 : >99 : <1 : <1	---	---
YHR104w	pIK29	---	---	---	---
YDR368w	pIK4	---	<1 : >99 : <1 : <1	---	---
YGL185c	pAKS1	---	<1 : 40 : <1 : 60	---	---
YNL274c	pIK13	---	<1 : 40 : <1 : 60	---	---
YPL275w	pIK18	---	---	---	---
YPL113c	pIK15	---	22 : 78 : <1 : <1	---	---
YLR070c	pIK23	---	---	---	---
YAL060w	pTM3	<1 : <1 : >99 : <1	<1 : <1 : 95 : 5	<1 : <1 : >99 : <1	<1 : <1 : 98 : 2
YGL157w	pIK7	<1 : <1 : >99 : <1	<1 : <1 : >99 : <1	<1 : <1 : >99 : <1	<1 : <1 : >99 : <1
YDR541c	pIK5	<1 : <1 : >99 : <1	---	<1 : <1 : >99 : <1	---
YGL039w	pIK6	<1 : <1 : >99 : <1	<1 : <1 : 90 : 10	<1 : <1 : >99 : <1	<1 : <1 : 97 : 3
YNL331c	pIK11	---	<1 : <1 : <1 : >99	---	<1 : <1 : <1 : >99
YCR107w	pIK10	---	<1 : <1 : <1 : >99	---	<1 : <1 : <1 : >99
YOL151w	pIK3	---	---	---	---
Yeast Cells	---	50 : <1 : 50 : <1	50 : 7 : 38 : 5	50 : <1 : 50 : <1	12 : 4 : 77 : 7

[a] <20% conversion after 24 hours[8]

Table 1-2. Comparison of reduction products for α-methyl and unsubstituted β-ketoesters

Yeast Gene	Plasmid	 **11a**	 **3a**	 **11b**	 **3b**
		anti-**12a** : *syn*-**12a** : *anti*-**13a** : *syn*-**13a**	**4a** : **5a**	*anti*-**12b** : *syn*-**12b** : *anti*-**13b** : *syn*-**13b**	**4b** : **5b**
YJR096w	pIK9	---	---	---	---
YDL124w	pIK8	---	---	---	---
YBR149w	pIK12	---	---	---	---
YOR120w	pIK30	---	<1 : >99	---	<1 : >99
YHR104w	pIK29	---	---	---	---
YDR368w	pIK4	---	<1 : >99	---	<1 : >99
YGL185c	pAKS1	---	---	---	---
YNL274c	pIK13	---	---	---	---
YPL275w	pIK18	---	---	---	---
YPL113c	pIK15	---	<1 : >99	---	16 : 84
YLR070c	pIK23	---	---	---	---
YAL060w	pTM3	<1 : <1 : >99 : <1	<1 : >99	<1 : <1 : >99 : <1	<1 : >99
YGL157w	pIK7	<1 : <1 : >99 : <1	<1 : >99	<1 : <1 : >99 : <1	<1 : >99
YDR541c	pIK5	<1 : <1 : >99 : <1	<1 : >99	<1 : <1 : >99 : <1	<1 : >99
YGL039w	pIK6	<1 : <1 : >99 : <1	<1 : >99	<1 : <1 : >99 : <1	<1 : >99
YNL331c	pIK11	---	---	---	23 : 77
YCR107w	pIK10	---	38 : 62	---	---
YOL151w	pIK3	---	<1 : >99	---	<1 : >99
Yeast Cells	---	50 : <1 : 50 : <1	70 : 30	50 : <1 : 50 : <1	>99 : <1

[a] <20% conversion after 24 hours[1,5] [b]Arbitrary assignment, *syn* product absolute configuration was not assigned

Table 1-3. Chemical shift difference between (*R*) and (*S*) MPA esters (Δδ*R*,*S*)

Compound	$dH_{2'}$	$dH_{1'}$	$dH_{1a'}$	dH_2	dH_{Me}	dH_3	dH_{4a}	dH_{4b}	dH_{5a}	dH_{5b}	dH_{Ω}	Alcohol Conf
anti-**13a**	*nm*[a]	0.28	0.24	0.10	0.17	0	-0.1	-0.13	na[b]	na[b]	-0.31	(*S*)
anti-**13b**	*nm*	0.21	0.26	0.11	0.19	0	-0.1	-0.13	-0.36	-0.36	-0.21	(*S*)

[a]Not measured [b]Not applicable

CHAPTER 2
SYNTHESIS OF AN APTAMER TARGET FOR RAPID SCREENING OF EXPOSURE TO CHEMICAL WARFARE AGENTS

Introduction

Chemical warfare (CW) began during World War I with the deployment of toxic gases such as phosgene, chlorine and dichloroethylsulfide (mustard gas).[25] More recently, CW was used by terrorists during the release of Sarin gas in Matsumoto City and Tokyo, Japan.[26] CW agents are toxic at low levels: patients admitted to the Nippon Medical Center after exposure to Sarin gas in the Tokyo incident had ingested estimated levels of 0.13 – 0.25 mg for a comatose patient (who never recovered) to 16-32 μg for patients who were sick but fully recovered.[27] The risk of serious injury, or death, from exposure to CW agents continues to the present day.

Rapid, accurate detection of CW agents is of vital importance in order to minimize their potentially devastating effects. Detection of exposure to CW agents relies upon detection of their degradation products since the intact agent is rapidly hydrolyzed *in vivo* (Figure 2-1).[28] Current methods for CW agent detection include GC / MS[28], HPLC / MS[29] or assays detecting inhibition of acetylcholine esterase (AChE).[30] Both of these techniques have disadvantages: GC / MS[28] or HPLC / MS[29] require at least 24 hours after sample acquisition, while AChE inhibition assays will not identify specific CW agents[30] and suffer from false positive results due to AChE inhibition from unrelated chemicals. Clearly, a need persists for rapid and accurate detection of CW agents.

Aptamers for CW Agent Detection

Aptamers are single stranded (ss)DNA or RNA oligonucleotides that can bind to targets due to their tertiary structure.[31] Aptamers are selected using an *in vitro* process called SELEX (Systematic Evolution of Ligands by EXponential enrichment) which begins with a large random pool of oligonucleotides and, through repeated rounds of selection against a target, identifies a

small number of oligonucleotides that tightly bind.[32-34] Aptamers can bind tightly to a wide range of targets ranging in size from live cancer cells (K_d = 0.80 nM)[35] to ethanolamine (K_d = 6 nM).[36,37]

We set out to isolate DNA aptamers that could bind to **18**, a biotinylated analog of CW agent hydrolysis product **17** (Figure 2-2). These aptamers could, in principle, be isolated since phosphonic acid **17** (n = 2) was detected at approximately 100 nM in the urine of a Japanese man one day after Sarin exposure in Matsumoto City.[38] Similar levels of **17** (135 – 25 nM) were found in the serum of victims 1.5 hours after hospital admittance in Sarin attacks in Tokyo and Matsumoto City.[29] These people became ill, but their exposure was not fatal.

We chose to biotinylate our analog to allow for easy separation of binding aptamers in solution using streptavidin coated magnetic nanoparticles.[39] After isolation of the DNA aptamers, a rapid colorimetric test for nerve agent exposure in the field would be developed by ADA Technologies using their proprietary technology.

Target Synthesis

Biotinylated phosphonic acid **18** was chemically synthesized in four steps starting from phosphonic dichloride **19**. The dichloride was converted to the mixed phosphonate ester **20** by sequential treatment with benzyl alcohol and propylene glycol.[40] Ester **20** was then oxidized to carboxylic acid **21** using TEMPO[41] followed by attachment of polyethylene glycol (PEG) amine **22** with polymer bound EDC[42] to form phosphonate **23**. After this step, some of amine **22** remained as an inseparable impurity. The benzyl ester was removed using H_2 / Pd[43] to give phosphonic acid **18** plus amine impurity **22** (Figure 2-3).

Aptamer Selection

We used a 76 nucleotide ssDNA template with a 40 nucleotide random sequence surrounded by forward and reverse priming regions. The primer sequences were chosen

according to Stoltenburg.[36] The forward primer contained a fluorescent (FAM) tag at its 5' end, while the reverse primer had a biotin tag at the 5' position (Figure 2-4). The fluorescent tag allowed for detection of the DNA and biotin allowed for purification and separation of double stranded DNA using streptavidin coated magnetic nanoparticles.

For the initial selection round, biotinylated target **18** was mixed with streptavidin (SA) coated magnetic nanoparticles (mnp's) and allowed to stand overnight at 4^{o} C before addition of the template strand. The resulting solution was then allowed to incubate at room temperature with gentle shaking for 30 minutes followed by removal of the nonbinding oligonucleotide containing supernatant from the mnp's. Bound DNA was eluted from the target by denaturation and the resulting ssDNA was amplified by PCR to give double stranded dsDNA containing a fluorescent tag and biotinylated tag on opposite strands. The dsDNA was then allowed to stand overnight with streptavidin coated mnp's; ssDNA containing the fluorescent tag was eluted by denaturation to give the starting material for the second selection round. Selection rounds 2-5 followed the same procedure as the first round (Figure 2-5).

Selection round 6 employed a counterselection step to determine if DNA was binding the mnp's or PEG impurity **22** instead of target **18**. The counterselection step used acetylated amine **23** as a mimic for PEG impurity **22** (Figure 2-6). The step was performed by binding acetylated amine **23** to streptavidin coated mnp's as described previously followed by incubation with isolated DNA from the selection process. As shown in Table 3, counterselection after round 6 resulted in a large decrease in fluorescence of the isolated DNA. Additionally, no DNA was isolated after PCR amplification.

Results and Discussion

A steady increase in fluorescence to blank should be observed after the initial selection round if DNA is tightly binding since larger quantities are recovered. We did not observe this

steady increase, allowing us to conclude that DNA was not specifically binding to target **18**, but instead to the polyethylene glycol impurity.

Generation of the PEG impurity could be avoided by attachment of the methyl phosphonic acid directly to the mnp's, as employed by Strehlitz et. al. for their preparation of ethanolamine modified mnp's.[36] This process would require incubation of phosphonic acid **24** with commercially available tosyl activated mnp's (Figure 2-7). These phosphonic acid modified mnp's could then be used for SELEX without any major modifications to our current procedure.

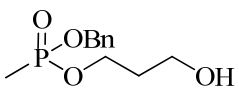
Finally, the similarity of the methyl phosphonic acid and phosphate esters in the DNA backbone should be noted. This could prevent DNA from binding tightly using either procedure since it is possible that DNA can not tell the difference between itself and the target. If this similarity is too great, then the detection of hydrolysis products of CW agents with DNA aptamers is not possible.

Experimental Procedures

General Experimental

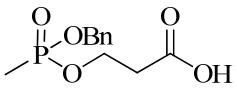
All chemicals and dNTP's were purchased from Sigma Aldrich or Fisher and used without further purification. Solvents were purified as described in the previous procedure. Biotinylated amine **22** was purchased from Pierce. *Taq* polymerase was purchased from New England Biolabs. All DNA was synthesized by IDT DNA Technologies. Fluorescence was measured using a Tecan Safire microplate reader with 384 well Corning plates.

Chemical Synthesis



20

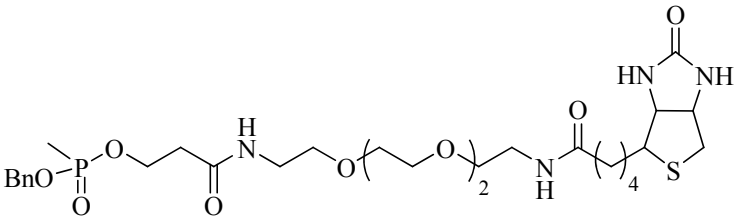
An oven dried flask was equipped with a stir bar and charged with 20 mL of CH_2Cl_2, methyl phosphonic dichloride (8.8 mmol, 0.8 mL) and triethylamine (17.6 mmol, 2.45 mL). Benzyl alcohol (8.8 mmol, 0.91 mL) was then added dropwise at room temperature so that a gentle reflux was maintained. The solution was stirred at room temperature for two hours before dropwise addition of propylene glycol (8.8 mmol, 0.64 mL) at room temperature. The reaction was then stirred overnight at room temperature. Triethylammonium salts were removed by filtration, the solvent was removed and the product purified by flash chromatography (EtOAc) to give the product (1.61 g, 75 % yield) as a colorless oil. ^{1}H NMR: ($CDCl_3$) δ 1.49 (d, 3H, J = 17.6 Hz), 1.82 (m, 2H), 3.72 (t, 2H, J = 5.7), 4.07 (m, 1H), 4.21 (m, 1H), 5.08 (d, 2H, J = 9.1 Hz), 7.4 (m, 5H); ^{31}P NMR: ($CDCl_3$) δ 33.82.



21

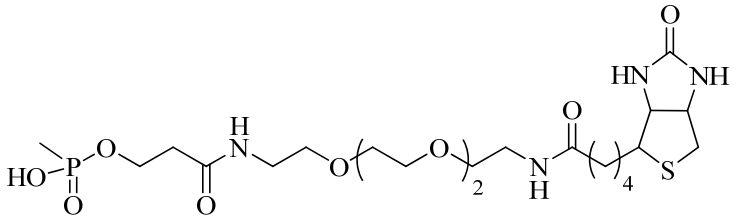
Aqueous 15 % $NaHCO_3$ (3 mL) was added to a solution of **20** (1.19 mmol, 0.29 g) in 25 mL of acetone and the solution was cooled to 0^o C. Sodium bromide (0.24 mmol, 0.024 g) and TEMPO (0.024 mmol, 0.004 g) were then added, followed by the addition of trichloroisocyanuric acid (2.38 mmol, 0.55 g) over a 20 minute period. The reaction was then stirred at room temperature for two hours before the addition of 1.5 mL of isopropanol. The mixture was filtered over Celite, concentrated under vacuum, and 8 mL of saturated Na_2CO_3 was added. The aqueous phase was washed with EtOAc (2 x 20 mL), then acidified with 1 M HCl and extracted again with EtOAc (3 x 20 mL). The combined organic layers from the second extraction were dried with $MgSO_4$ and concentrated under vacuum to yield the product as a semicrystalline solid (crude yield 0.26 g, 85 %) which was used without purification in the next

step. ^{1}H NMR: ($CDCl_3$) δ 1.50 (d, 3H, *J* = 17.8 Hz), 2.68 (t, 2H, *J* = 6.12 Hz), 4.26 (m, 2H), 2.08 (dd, 2H, *J* = 3.9, 9 Hz), 7.36 (m, 5H). ^{31}P NMR: ($CDCl_3$) δ 36.87.



23

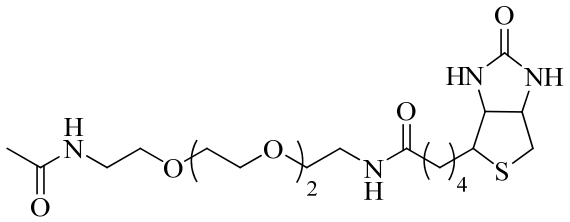
Carboxylic acid **21** (0.06 mmol, 16 mg) and PEG biotinylated linker (0.06 mmol, 25 mg) were combined in an oven dried flask that contained 2 mL of CH_2Cl_2 and 2 mL of DMF. Polymer bound EDC (100 mg) was then added and the reaction was stirred overnight at room temperature. The solvents were then evaporated and the crude product was purified by flash chromatography (5 : 1 EtOAc : MeOH) to give the product (crude yield 0.025 g, 65 % yield) plus some of the PEG biotinylated linker as an inseparable impurity. ^{1}H NMR: (CD_3OD) δ 1.26 (m, 3H), 1.44 (m, 2H), 1.52 (d, 3H, *J* = 15 Hz), 1.64 (m, 4H), 2.22 (t, 2H, *J* = 7.4 Hz), 2.56 (t, 2H, *J* = 6.0 Hz), 2.70 (m, 1H), 2.93 (m, dd, *J* = 5.1, 12.7 Hz), 3.20 (m, 2H), 3.37 (m, 10H), 3.53 (m, 5H), 3.62 (m, 11H), 4.28 (m, 3H), 4.5 (dd, 1H, *J* = 4.5, 8.1 Hz), 5.07 (dd, 2H *J* = 2.3, 8.5 Hz), 7.4 (m, 6H); ^{31}P NMR: (CD_3OD) δ 33.8.



18

Biotinylated phosphonate **23** (0.025 g, 0.039 mmol) was added to a flask containing 15 mL of MeOH. A spatula tip full of Pd on Carbon was then added to the flask and the mixture was hydrogenated at room temperature under a balloon of hydrogen overnight. The mixture was then

filtered over Celite and MeOH was removed under reduced pressure. The product was then lyophilized to give yellow crystals (0.022 g, 94 % yield). ^{1}H NMR: (D_2O) δ 1.10 (m, 6H), 1.30 (s, 1H), 1.50 (m, 5H), 1.75 (m, 2H), 1.95 (s, 1H), 2.15 (m, 1H), 2.42 (t, 2H, *J* = 5.7 Hz), 2.68 (s, 1H), 2.84 (s, 1H), 3.2 (m, 9H), 3.52 (m, 6H), 4.57 (s, 1H). ^{31}P NMR: (D_2O) δ 28.20.
(+) ESI-MS: 584 (M + 16, impurity from oxidation of biotin sulfur to sulfoxide), 568 (m/z for biotinylated methyl phosphonic acid **18**, most abundant compound), 418 (m/z for biotin linker impurity **22**).



23

PEG biotinylated linker (12 mg, 0.028 mmol), acetic anhydride (5.3 μL, 0.056 mmol) and N,N-dimethylaminopyridine (1 small crystal) were combined in an oven dried flask that contained 6 mL of CH_2Cl_2 at room temperature and stirred overnight at room temperature. The solvent was removed under reduced pressure and the resulting solid purified by flash chromatography (5 : 1 EtOAc : MeOH) to give **23** (6.4 mg, 50 % yield). ^{1}H NMR: ($CDCl_3$) δ 1.45 (m, 2H), 1.70 (m, 4 H), 1.95 (s, 3H), 2.25 (t, 2H, *J* = 6.9 Hz), 2.75 (m, 1H), 2.92 (dd, 1H, *J* = 5.1, 12.9 Hz), 3.15 (m, 1H), 3.44 (m, 4H), 3.57 (t, 4H, *J* = 5.1 Hz), 3.64 (s, 6H), 4.33 (m, 1H), 4.51 (m, 1H), 5.50 (s, 1H), 6.49 (s, 1H), 6.60 (s, 1H), 6.76 (s, 1H).

PCR Amplification

The reactants for each PCR round were 0.2 mM dNTP's, 1 μM forward primer, 1 μM reverse primer, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl pH 8.8, 10 mM $MgSO_4$, 0.1% Triton X-100. PCR conditions were 5 minutes at 94°C and 30 cycles of 1 min at 94°C, 1 min at 47°C, 1 min at 72°C, then 10 minutes at 72°C after the last cycle. 20 units of Taq polymerase (5

U / μL) were used for a 100 μL PCR; 10 units were initially added via hot start methodology and 10 units were added after 15 cycles. Electrophoresis on a 2% agarose gel with Hae III phix174 markers were used to determine if the correct size of DNA was produced.

Magnetic Nanoparticles

Magnetic nanoparticles were synthesized by Joshua Smith at the University of Florida. An aqueous mechanically stirred solution of ammonium hydroxide (2.5%), ferric chloride hexahydrate (0.5 M), ferrous chloride tetrahydrate (0.25 M) and HCl (0.33 M) was stirred for 10 minutes at 350 RPM. The iron oxide nanoparticles were then washed with water three times and ethanol once. The MNPs were dispersed in an ethanol solution containing ~1.2% ammonium hydroxide at a final concentration of ~7.5 mg / mL to a final volume of ~6 mL.

The magnetite core particles were coated with silica by adding 200 μL tetraethylorthosilicate (TEOS) to the ethanolic solution and sonicating for 90 minutes at room temperature. Additional TEOS (10 μL) was introduced and sonication was continued at room temperature for an additional 90 minutes to post-coat the nanoparticles. The sample was washed three times with ethanol. To introduce surface carboxyl groups, 80 μL of carboxy-silane (N-(trimethoxysilylpropyl)ethylenediamine, triacetic acid trisodium salt, 45% in water) was added to 1 mL of silica-coated magnetic nanoparticles (10 mg / mL suspension in 10 mM phosphate-buffered saline, pH 7.4) and the reaction was vortexed for 4 hours at room temperature. The particles were then washed three times with 10 mM phosphate-buffered saline and stored at room temperature.

To prepare for streptavidin coupling, a 1 mg sample of carboxyl-modified magnetic nanoparticles was washed three times with 250 μL aliquots of 0.5 mM MES, pH 5.0. Protein immobilization was carried out by adding 50 μL of a 20 mg / mL EDC solution to the washed

nanoparticles in 250 μL of 0.5 mM MES, pH, 5.0 and incubating at room temperature for 15 minutes before 100-300 μg of streptavidin was added to the reaction mixture. The suspension was incubated for 2-4 h with continuous vortexing. The streptavidin-coated nanoparticles were magnetically extracted and washed three times with 500 μL aliquots of 20 mM Tris-Cl, 5 mM $MgCl_2$, pH 8, then resuspended at a final concentration of 2 mg / mL in the same buffer and stored at 4°C.

Round 1 of SELEX

A 500 μL eppendorf reaction tube containing 3 x 10^{-18} mole of target biotinylated phosphonic acid (30 μL from a 1 x 10^{-12} M stock solution), 20 μL of streptavidin coated magnetic nanoparticles and 380 μL of buffer was allowed to stand overnight at 4°C. 3 nanomoles of the template sequence was then added (70 μL from a 1μg / μL stock solution) and the reaction was allowed to incubate at room temperature for 30 minutes with gentle shaking. (Before addition, DNA was denatured and renatured by heating to 80°C for 10 min and then cooling on ice for 10 minutes).

The eppendorf tube was then attached to a magnet and allowed to stand for 5 minutes. The supernatant was removed, 200 μL of fresh buffer was added, the tube was gently vortexed, attached to a magnet and allowed to stand for 5 minutes before removing the supernatant. This procedure was performed three total times.

The DNA bound to the phosphonic acid was eluted by adding 100 μL of fresh binding buffer to the eppendorf tube, attaching the tube to a magnet, binding for 5 minutes and then heating to 94°C with shaking for 8 minutes followed by immediate removal of the supernatant while the solution was still hot. This procedure was performed two total times. After elution, DNA was precipitated with EtOH and 4 M NaCl (EtOH = 2.5 x total volume buffer used, 4 M NaCl = 0.025 x total volume buffer used), and allowed to stand overnight at -20°C. The solution

was then microfuged for 20 minutes and the supernatant was removed to give the eluted DNA. The eluted DNA was PCR amplified using the conditions listed in the general experimental.

After amplification, the ds DNA was purified and separated into ss DNA by adding 20 μL of MNP's to the PCR vial and allowing to stand at 0^{o}C overnight. The tube was then attached to a magnet, allowed to stand for 5 minutes and the supernatant removed. 100 μL of fresh buffer was added, the tube was vortexed, attached to a magnet and allowed to stand for five minutes followed by removal of the supernatant. This procedure was performed three total times. After washing was complete, 100 μL of fresh buffer was added, the tube was attached to a magnet, bound for 5 minutes, heated to 94^{o} C for 8 minutes while bound to a magnet with shaking and the supernatant was removed while still hot. This procedure was performed two total times to give the purifed ss FAM labelled DNA in 200 total μL of buffer. The presence of DNA was monitored by fluoresence and by visualization of the amplified DNA after gel electrophoresis.

Rounds 2-5 of SELEX

For subsequent selex rounds the entire PCR amplified and purified ss FAM labelled DNA from the previous round was used. For example, selex for round 2 contained 200 μL of purified DNA solution, 250 μL of buffer and 30 μL of the phosphonic acid from a 1×10^{-12} M stock solution and 20 μL of magnetic nanoparticles.

Round 6 of Selex Plus Counterselection Step

Round 6 introduced a counterselection step to ensure that DNA was binding to the phosphonic acid and not to the magnetic nanoparticles or biotinylated polyethylene glycol linker impurity **1**. The selex procedure was carried out as usual to give 200 μL of solution after elution from the phosphonic acid. To this 200 μL solution was added 50 μL of a solution which contained 20 μL of magnetic nanoparticles and 30 μL of a 1×10^{-12} M solution of acetylated PEG linker (The acetylated PEG linker and magnetic nanoparticles were allowed to bind

overnight at 0^{o}C before addition). The entire solution was shaken gently for 30 minutes, the tube was attached to a magnet and the supernatant removed. 100 μL of buffer was then added, the tube was gently vortexed, attached to a magnet and the supernatant was removed. The recovered solution was precipitated and subjected to PCR amplification conditions as described previously.

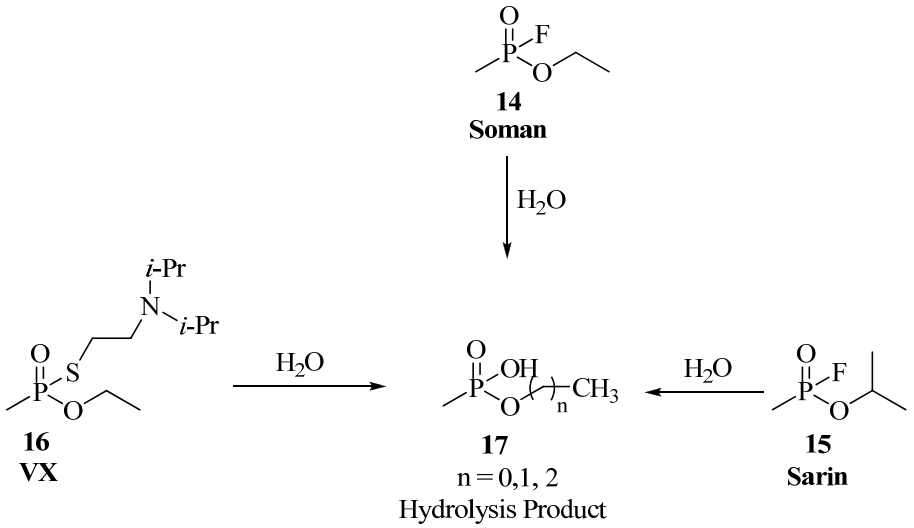


Figure 2-1. Hydrolysis product of various CW agents

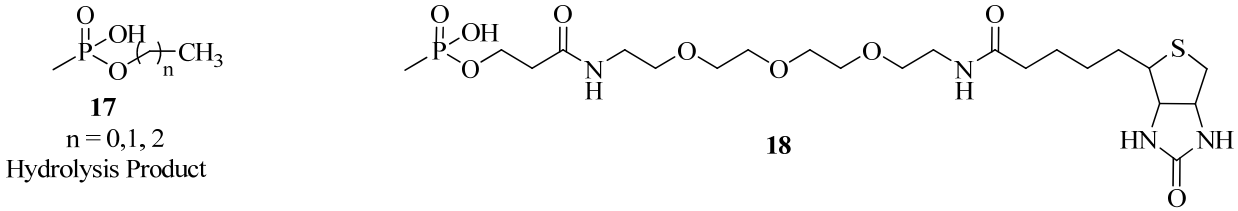


Figure 2-2. Chemical warfare hydrolysis products vs. biotinylated analog

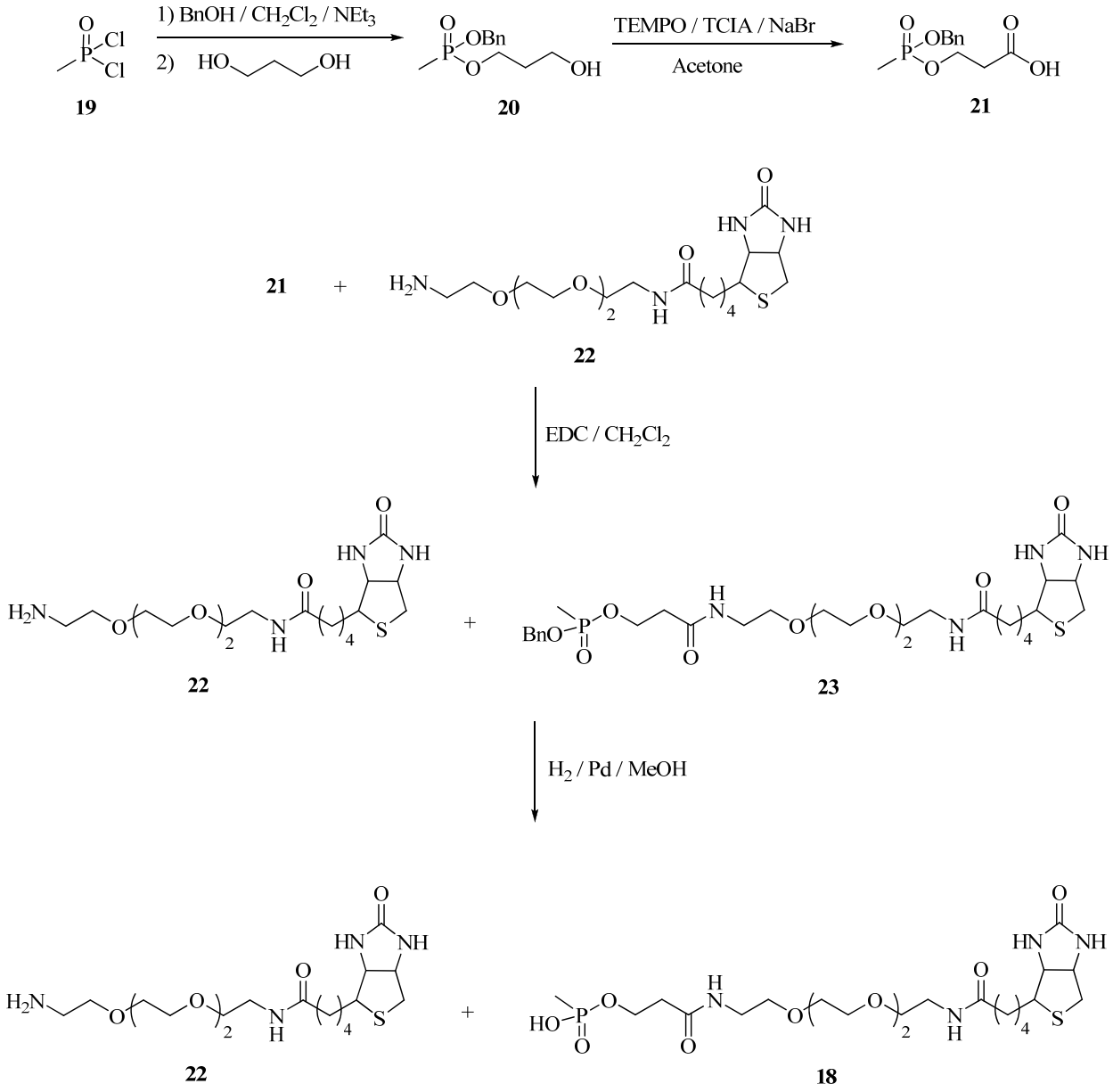


Figure 2-3. Synthesis of biotinylated CW agent hydrolysis product

Template Strand

5’-ATA CCA GCT TAT TCA ATT N_{40} AGA TAG TAA GTG CAA TCT-3’

Forward Primer

5’-FAM-ATA CCA GCT TAT TCA ATT-3’

Reverse Primer

5’-BIOTIN-AGA TTG CAC TTA CTA TCT-3’

Figure 2-4. Template strand, forward and reverse primers

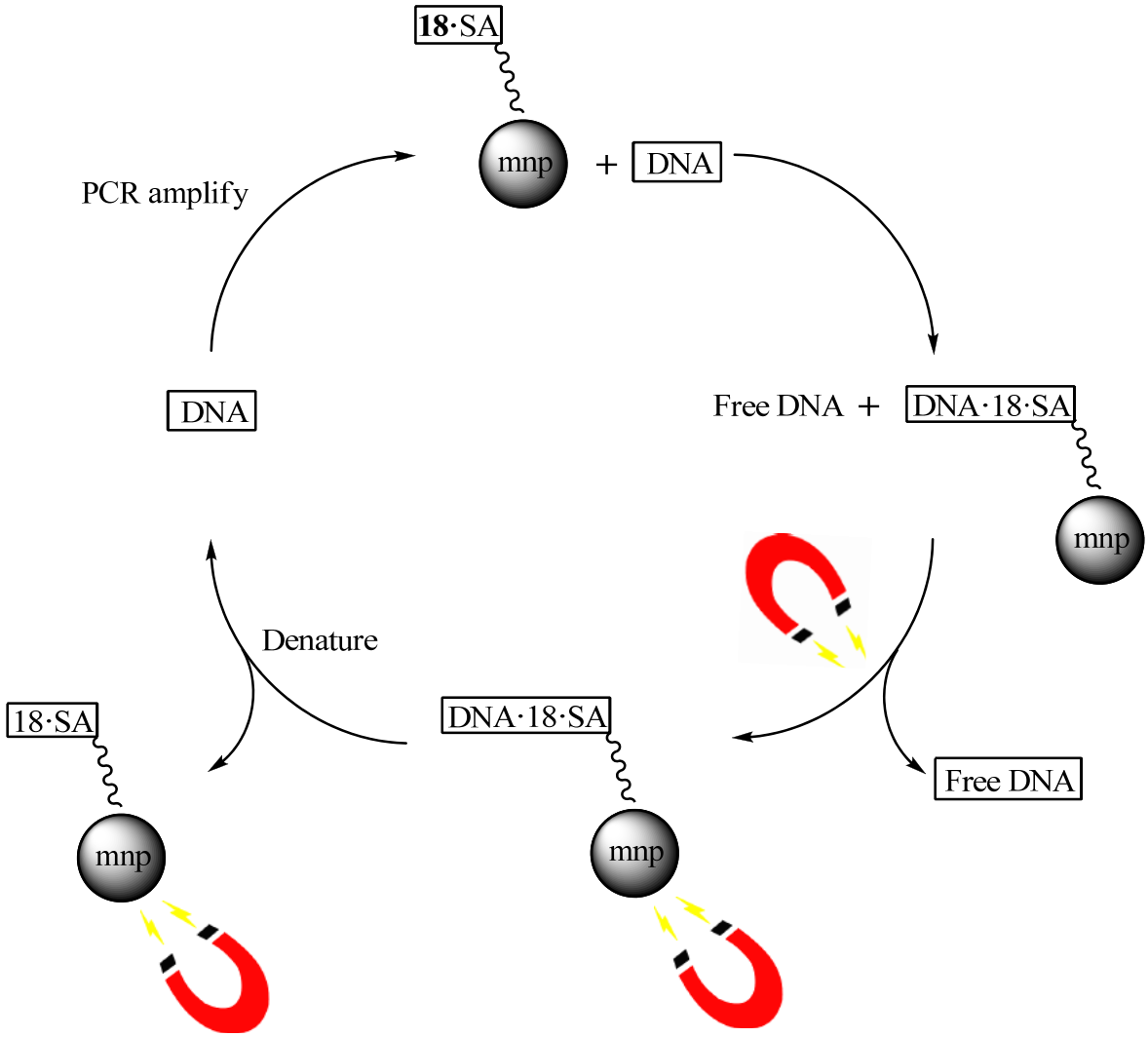
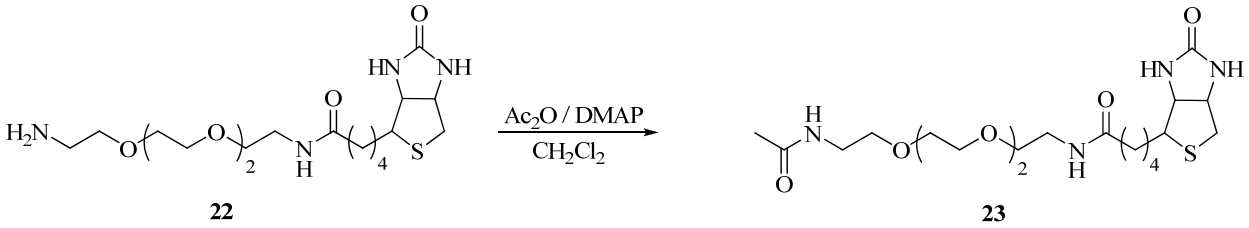


Figure 2-5. Procedure for SELEX



22 23

Figure 2-6. Synthesis of acetylated PEG impurity

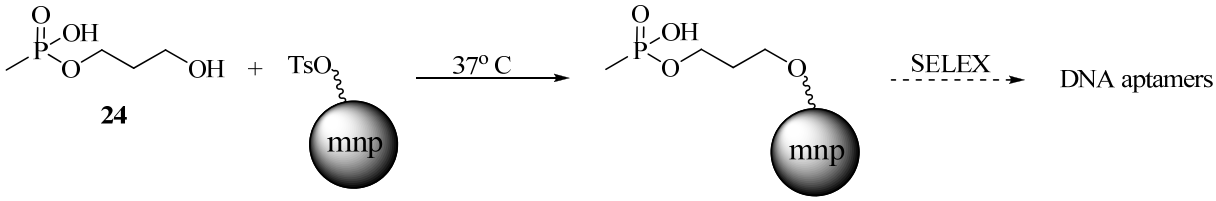


Figure 2-7. Direct attachment of methyl phosphonic acid to mnp's

Table 2-1. Selection and counterselection fluorescence data

Round	Ratio of Fluorescence to Blank
1	320
2	11
3	267
4	11
5	21
6	173
6 + Counterselection	14

CHAPTER 3
CHEMOENZYMATIC ROUTE TO FLUORINATED PHOSPHONIC ACID CARBOHYDRATES OF DEFINED STEREOCHEMISTRY

Introduction

The α-Fluorophosphonates

Phosphate esters and anhydrides are widespread in biological systems. These compounds interact with enzyme binding sites through electrostatic, hydrogen bonding and dipole-dipole interactions.[24] The frequent presence of phosphate esters/anhydrides has fueled research to synthesize molecules that contain these useful functional groups. Unfortunately, phosphate esters/anhydrides are susceptible to hydrolysis *in vivo* by phosphatases, limiting their potential as successful drugs.

Phosphonates, which differ from phosphates by methylene replacement of one bridging oxygen, have been envisioned as nonhydrolyzable mimics of phosphate esters/anhydrides. Blackburn et al.[44] proposed that α-fluorinated phosphonates might serve as superior phosphate analogs because of increased methylene polarity (Figure 3-1). α-Fluorinated phosphonic acids can also be considered as 'isopolar' mimics of phosphates due to the reduced p*K*a of the free hydroxyl groups (Figure 3-2).[45] Other reasons postulated for the superiority of fluorinated phosphonates include increased bridging atom dihedral angle[46] and the possibility for C-F···H-X hydrogen bonding (Figure 3-3).[47,48]

Routes to α-Monofluorophosphonates

α-Fluorophosphonates are made using various techniques. Blackburn et al.[49] and Prestwich et al.[50] used the Horner-Wadsworth-Emmons (HWE) reaction of fluorinated bisphosphonate **36** to build vinyl α-fluorophosphonates **37**. The vinyl phosphonates can be converted to alkyl α-fluorophosphonates by reduction with Pd / C at atmospheric pressure (Figure 3-4).[50]

HWE,[51] Peterson[52] and alkylation[53] reactions with dibromofluorophosphonate **40** were used by Savignac to make vinyl and alkyl α-fluorophosphonates. Phosphonate **40** is the Arbuzov product of triethyl phosphite and tribromofluoromethane.[54] Savignac's strategy generally relied upon double lithium halogen exchange using *n*-BuLi with in situ trimethylsilyl (TMS) protection/stabilization of the resulting lithiated anion before reaction with an aldehyde or alkyl halide (Figure 3-5).

Electrophilic fluorination using N-fluorobenzenesulfonimide (NFSI) **52** or Selectfluor **53** has been used for the selective fluorination of phosphonates, phosphonoacetates and β-sulfonophosphonates. NFSI and Selectfluor[55] are stable, solid, easily handled sources of fluorine that can be used without any special precautions or special training. McKenna et. al[56] used Selectfluor to build α-fluorophosphonoacetates while Wnuk et. al[57] used Selectfluor to make α-fluorosulfones. The choice of counterion (Na^+ or K^+) can be important when optimizing electrophilic fluorinations.

Finally, (diethylamino) sulfur trifluoride (DAST) **54** has been employed for the nucleophilic displacement of α-hydroxyphosphonates by fluorine. DAST was used by Prestwich et al.[58] for their synthesis of analogs of lysophosphatidic acid. DAST is thermally unstable, so reactions must be conducted at room temperature or below (Figure 3-6).[59]

Methods for Stereochemical Control of α-Monofluorophosphonates

Optically pure α-fluorophosphonates are currently made using chiral Lewis Acid or organometallic complexes. Joergensen et al.[60] used chiral Lewis acid complex **57** to fluorinate α-substited β-ketophosphonates with good enantiomeric excess while Sodeoka et al.[61] used chiral Pd complex **60** to carry out enantioselective fluorination (Figure 3-7). However, neither method was reported to selectively fluorinate α-unsubstituted phosphonates.

The Importance of Stereochemical Control of α-Monofluorophosphonates

O'Hagan and coworkers synthesized *sn*-glycerol-3-phosphate (G3P) analogs **61-64** (Figure 3-8) and assayed them for activity against glycerol-1-phosphate dehydrogenase (G1DH) by monitoring the rate of NADH formation (Figure 3-9).[46] O'Hagan found that α-monofluorinated phosphonic acid **63** was a substrate for the enzyme. In fact, both methylene **62** and α-monofluoro **63** were better than the natural substrate, **61**. O'Hagan also examined the diastereomers of **63** to see if G1DH preferred one, and discovered that one was consumed 20% faster (Figure 3-10). Unfortunately, he was not able to determine the configuration of the better substrate or detect any accumulation of α-hydroxyketone product **65**.

Berkowitz et al.[62] constructed phosphonate analogs **66-69** of glucose-6-phosphate (G6P) (Figure 3-11).[63] Berkowitz tested these G6P analogs as substrates for glucose-6-phosphate dehydrogenase (G6PDH) (Figure 3-12), and found that the analogs with the highest (**68**) and lowest (**67**) K_m varied only by the stereochemistry of the α-fluorine. In fact, an order of magnitude separated the binding of the two compounds. Clearly, further research on stereochemical control of α-fluorinated phosphonates was warranted from the findings of O'Hagan and Berkowitz.

Aldolases in Synthetic Chemistry

Aldolases are enzymes that selectively catalyze the aldol reaction between a donor substrate and acceptor aldehyde to create two new stereocenters. Aldolases are classified according to their reaction mechanism: class I use a lysine residue in the active site to create a donor substrate enamine which then attacks an acceptor aldehyde, class II use Zn^{2+} to stabilize a donor enolate within the active site before attack on the acceptor aldehyde (Figure 3-13). The donor substrate for most class I and class II aldolases is dihydroxyacetone phosphate (DHAP), **72**.

Aldolases have been studied as tools for asymmetric organic synthesis using non-natural phosphonic acid donor substrate **80**. Stribling[63] discovered **80** could substitute for natural phosphate substrate **72** in the fructose 1,6-bisphosphate aldolase (FruA) catalyzed aldol reaction with D-glyceraldehyde 3-phosphate **81** to make phosphonic acid carbohydrate **82** (Figure 3-14).

Fessner et al.[64] synthesized phosphonic acid sugars **83** and **84** using substrate **80** with aldolases from class I and class II, allowing him to make different diastereomeric products from the same starting materials (Figure 3-15). Thus, we reasoned that optically pure fluorinated DHAP mimics **85a-b** could be used with aldolase to synthsize optically active fluorinated phosphonic acid carbohydrates (Figure 3-16).

Selective Reduction of α-Fluoro-β-Ketophosphonates

Our initial route to optically pure DHAP mimics **85a-b** relied upon selective reduction of α-fluoro-β-ketophosphonate **88**. The corresponding alcohol **89** would then be deoxygenated using standard Barton-McCombie conditions, followed by acetonide deprotection and selective oxidation of the secondary alcohol to give phosphonate ester **92**. Deprotection of the phosphonate ester would give phosphonic acid DHAP mimics **85a-b** (Figure 3-17).

α-Fluoro-β-ketophosphonate **88** was made in two steps via reaction of dimethyl methylphosphonate with acetonide methyl ester **95**[65] followed by electrophilic fluorination of β-ketophosphonate **97** with Selectfluor.[57] Methyl ester **95** was made by selective protection of D-mannitol[66] followed by $NaIO_4$ cleavage of diol **94**[65] and immediate oxidation of the resulting aldehyde to the methyl ester using Br_2 / $NaHCO_3$ / MeOH. Fluorination of **97** gave low yields of monofluorinated compound in addition to a difficult chromatographic separation of difluorinated and monofluorinated products (Figure 3-18).

The α-fluoro-β-ketophosphonate **88** was then reduced by $NaBH_4$ to give racemic standard **98**. Compound **88** was then screened against our library of purified β-ketoreductases from

Baker's Yeast using the same conditions as listed in Figure 1-7. The reactions were monitored with gas chromatography. Unfortunately, none of the reductases accepted the ketone. We next attempted to modify the acetonide protecting group of α-fluoro-β-ketophosphonate **88** with the less sterically demanding carbonate **99** (Figure 3-19).

Since quantities sufficient for further synthesis of α-fluoro-β-ketophosphonate **88** were difficult to obtain it was decided not to install the carbonate by acetonide deprotection / carbonate reprotection of **88** to give carbonate **100**. Instead, acetonide deprotection of β-ketophosphonate **97**, which was available on a multigram scale, using Dowex H^+ resin[67] to give diol **101** followed by carbonate formation with triphosgene[68] to access carbonate **102** on a gram scale. Unfortunately, all attempts to fluorinate **102** yielded only difluorinated product **103** and recovered starting material (Figure 3-20 and Table 3-1). The synthetic route to α-fluorinated DHAP mimics of defined stereochemistry via reduction of an α-fluoro-β-ketophosphonate followed by Barton-McCombie deoxygenation of the resulting alcohol was not further pursued.

Selective Reduction of α-Fluorovinylphosphonates

We next hypothesized that DHAP mimics **85a-b** could be synthesized by selective reduction of α-fluorovinylphosphonates **104a-b** using our library of enone reductases cloned and purified by Despina Bougioukou (Figure 3-21).[5,69] Our route to α-fluorovinylphosphonates used Horner-Wadsworth-Emmons (HWE) methodology to install the fluorinated alkene.

The initial synthesis of **104a-b** used the HWE reaction of fluorinated bisphosphonate **108** with protected aldehyde **112** to make the *E* isomer of protected phosphonate **113** as the sole product.[49] The fluorinated bisphosphonate **108** was constructed in two steps by intial synthesis of methylene bisphosphonate **107** from diethyl methylphosphonate **106** and diethyl chlorophosphate[70] followed by electrophilic fluorination with Selectfluor.[71] The protected aldehyde was made by ketalation of 1,3-dihydroxyacetone **109** followed by mono acetyl

protection of the 1,3-diol with *Pseudomonas fluorescens* Lipase[72] and subsequent Swern oxidation to aldehyde **112** (Figure 3-22).

This synthetic route proved laborious due to difficulty purifying fluorinated bisphosphonate **108** via flash chromatography or fractional distillation. Additionally, it was found that the HWE reaction of crude **108** with aldehyde **112** gave an inseparable mixture of products. However, HWE reaction of the in situ generated lithiated **108a** gave vinyl α-fluorinated phosphonate **113** in one step starting from α-diethyl-dibromofluoromethyl phosphonate **40** (Figure 3-23).[51]

The dibromofluoromethyl phosphonate was generated by the Arbuzov reaction of triethyl phosphite and tribromofluoromethane.[54] This reaction sequence allowed us to completely circumvent the purification problems associated with α-fluorobisphosphonate **108**. After considerable experimentation, it was found that deacetylation of **113** with Amberlyst A-26 resin[72] followed by ketal deprotection with Montmorillonite clay[73] afforded α-fluorinated vinyl phosphonate **104a** in decent yield (Figure 3-24).

Racemic standard **115**, the reduction product of α-fluorinated vinylphosponate **104a-b**, was prepared before screening vinyl phosphonate **104a** against our library of purified en-reductases to allow for reaction monitoring by gas chromatography. Synthesis of the racemic standard began with reduction of α-fluorinated phosphonate **113** with PdOH / C followed by acetyl deprotection using Amberlyst A-26 and deketalation with Montmorillonite clay (Figure 3-25). This route proved cumbersome due to difficulty separating the reduction products of **113**, but served to make sufficient quantities of crude **115** for GC analysis.

α-Fluorinated phosphonate **104a** was then screened against our library of en-reductases using a glucose-6-phosphate / glucose-6-phosphate dehyrogenase cofactor regeneration system;

the screenings revealed that starting material was consumed, but no product could be detected by GC (Figure 3-26). In order to identify the reaction product(s), we ran small scale enzymatic reactions in D_2O with stoichiometric quantities of NADPH.

The ^{19}F NMR after four hours showed complete consumption of starting material with the production of a single peak corresponding in chemical shift to inorganic fluoride (Figure 3-27). Presumabely, the fluorine was eliminated during reduction of the α-fluorovinylphosphonate via the mechanism in Figure 3-28 to give unfluorinated vinyl phosphonate **119**.

To support this mechanism, we synthesized vinyl phosphonate **119** via the same reaction sequence used to make fluorinated vinyl phosphonate **104a**. Thus, bisphosphonate **107** was condensed with aldehyde **112** to give protected vinyl phosphonate **113**. Acetyl deprotection with Amberlyst A26 followed by deketalation with montmorillonite clay gave **119** (Figure 3-29). Unfluorinated vinyl phosphonate **119** was not observed as a product of the enzymatic reduction of fluorinated vinyl phosphonate **104a** with stoichiometric quantities of NADPH (reaction monitored by ^{31}P NMR), but **119** was detected as an inseparable by-product from the HWE chemical synthesis of racemic fluorinated phosphonate **115** during ketal deprotection (Figure 3-30).

Synthesis of Racemic α-Fluorinated Phosphonic Acids

We decided to optimize the synthesis of racemic α-fluorinated phosphonate **115** before further investigation on the construction of α-fluorinated phosphonic acids of defined stereochemistry **85a-b** to determine if the fluorinated phosphonic acids are aldolase substrates. Our HWE route to **115** was not sufficient for this purpose, but recent literature reported that terminal alkenes can be converted to α-hydroxyketones in one step using Ruthenium or $KMnO_4$ oxidants.

Thus, unsaturated phosphonate **122** was made via double lithium halogen exchange / in situ trimethylsilyl (TMS) protection of dibromofluorophosphonate **40** followed by nucleophilic attack on allyl iodide to give the TMS protected unsaturated phosphonate.[53] This product was not isolated, but instead deprotected in the same pot using lithium ethoxide to give pure **122** after flash chromatography purification (Figure 3-31).

Unfortunately, oxidation of **122** with $KMnO_4$ yielded a mixture of α-hydroxyketone **115** plus unfluorinated vinyl phosphonate **119** in roughly a 1 : 1 ratio. Phosphonate deprotection of this mixture led to multiple unidentified phosphorus containing products (Figure 56). Oxidation with RuO_4 gave no defluorination, but instead yielded carboxylic acid **123** in low yield (Figure 3-32).

We then hypothesized that deprotection of unsaturated phosphonate **122** followed by alkene oxidation could lead to racemic phosphonic acid **124** (Figure 3-33). Phosphonate **122** was smoothly deprotected with TMSBr followed by hydrolysis of the TMS protected phosphonic acid with water to give phosphonic acid **125**.[73] However, $KMnO_4$ alkene oxidation was not successful and RuO_4 oxidation gave carboxylic acid **126** as the only reaction product (Figure 3-34). Our results with alkene oxidation revealed that this was not a method for synthesis of racemic α-fluorophosphonic acids.

Our next route to racemic α-fluorophosphonic acids sought to selectivly oxidize the secondary alcohol of diol **120** to α-hydroxyketone **118** using glycerol-1-phosphate dehydrogenase (G1DH) (Figure 3-35). For this procedure we planned to use NADH oxidase from *Lactobacillus sanfranciscensis* for cofactor regeneration.

Our route to diol **63** started from the $NaIO_4$ cleavage of acetonide protected D-mannitol **94** followed by immediate reduction of the resulting aldehyde to acetonide protected alcohol

127.[76] This alcohol was converted to triflate **128**[77] and then condensed with phosphonate **40** to give acetonide protected phosphonate **129** in decent yield.[46] Both protecting groups of **129** were removed by initial treatment of the neat phosphonate with TMSBr followed by addition of water for silyl ether cleavage and acetonide deprotection to give diol **63** as a 0.8 : 1 diastereomeric mixture as determine by ^{19}F NMR (Figure 3-36).[46]

Phosphonic acid **63** (6 mg / 0.53 μmol) was combined with G1DH (80 Units), NADH oxidase from *L. sanfranciscensis* (80 U) and NAD^+ (50 mg / 0.08 mmol) in 50 mM phosphate buffered D_2O and stirred at room temperature for 24 hours. The diastereomeric ratio changed during this time period from 0.8 : 1 to 0.4 : 1, indicating that starting material was consumed. We are currently unsure if product was formed, but this data will be obtained by monitoring the reaction by ^{13}C NMR to observe ketone formation or by mass spectrometry to observe molecular weight change.

Results and Discussion

While logical, our routes to α-fluorinated DHAP mimics of defined stereochemistry were not successful. We initially believed that our library of purified reductases from Baker's yeast could accept acetonide protected α-fluoro-β-ketophosphonate **88** due to our previous success with sterically similar furan derivative **130** (Figure 3-37). Unfortunately, acetonide protected phosphonate **88** proved too large for our enzymes, and installation of a less sterically demanding protecting group resulted in difluorinated products.

Our enone reductase route for asymmetric synthesis of α-fluorinated DHAP mimics via vinyl α-fluorophosphonates was not viable due to the unexpected elimination of fluorine after enzymatic reduction. Chemical synthesis of phosphonate **115** or phosphonic acid **65** as racemic mixtures via olefin oxidation also did not produce the pure compound due to fluorine

elimination. But, these methods gave us valuable insight about the sensitive nature of these compounds; any future syntheses must avoid the facile loss of fluorine.

Our current route for the synthesis of α-fluorinated DHAP mimics as a racemic mixture appears promising. Additional data, obtained from ^{13}C NMR or mass spectrometry, must be collected to ensure α-hydroxyketone formation from oxidation of diol **63**, since we currently are unsure of the fate of consumed starting material. If the oxidation is successful, then the α-fluorinated DHAP mimic will not be isolated, but instead reacted in situ with aldolase to form diastereomeric mixtures of α-fluorophosphonic acid carbohydrates. The in situ reaction of α-hydroxyketone **65** should minimize defluorination since the enolizable ketone will be converted to the lactol by aldolase (Figure 3-38).

Future Work

Future work will chemoenzymatically synthesize optically pure α-fluorophosphonic acids **85a-b** from optically pure α-hydroxyphosphonates **133a-b** (Figure 3-39). Alpha-hydroxyphosphonates **133a-b** have been made previously from (*S*)-malic acid **134** by Prestwich et al. as diastereomeric mixture **135**.[58] We plan to separate the diastereomers via lipase catalyzed kinetic resolution as shown by Backvall et al. for α-hydroxyphosphonate **136** (Figure 3-40).[78]

Nucleophilic displacement of kinetically resolved alcohol **132a** with diethylaminosulfur trifluoride (DAST) will give optically pure α-fluorophosphonate **140b**.[62] Or, the alcohol will be converted to the triflate followed by nucleophilic displacement with cesium fluoride[79] or tetrabutylammonium fluoride (TBAF)[80] to give **140b**. Deprotection of **140b** as previously described will give α-fluorophosphonic acid **63b** (Figure 3-41).

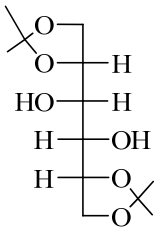
Diastereomer **63a** will be obtained by removal of the acteyl group under neutral conditions[81] followed by nucleophilic fluorination and deprotection (Figure 3-42). Optically

pure α-flurophosphonic acids **63a-b** will then be selectively oxidized and reacted in situ with aldolase to make α-fluorinated phosphonic acid carbohydrates of defined stereochemistry.

Experimental Procedures

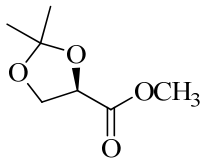
Materials

All organic chemicals were purchased from Sigma or Fisher and used without further purification. THF was distilled from Sodium metal. Flash chromatography was monitored by TLC with staining of plates by $KMnO_4$.



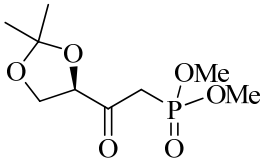
94

D-mannitol (28 g / 0.15 mol), dry DMSO (30 mL), 2,2-dimethoxypropane (46 mL / 0.38 mmol), and *p*-TsOH (0.5 g / 2.63 mmol) were added to an oven dried flask and stirred under Argon overnight. The solution was then poured into 3% $NaHCO_3$ (150 mL) and the resulting aqueous solution extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with water (2 x 20 mL) and brine (1 x 20 mL), dried over $MgSO_4$, and the solvent removed under reduced pressure. The resulting product was placed under high vacuum for 1.5 hours to remove residual solvent and the crude product recrystallized from heptane : $CHCl_3$ (10 : 1 volume / volume based on crude mass of product) to give 22 g of the pure product as white needles (56% yield).[82] Melting point 118 – 120° C; ^{1}H NMR: ($CDCl_3$) δ 1.36 (s, 6H), 1.43 (s, 6H), 2.80 (m, 2H), 3.75 (m, 2H), 3.8-4.3 (m, 6H); ^{13}C NMR: ($CDCl_3$) δ 109.3, 75.8, 70.9, 66.7, 26.7, 25.2.



95

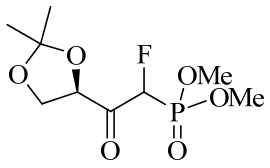
Acetonide **94** (6.07 g / 23.15 mmol) was added to 150 mL of CH_2Cl_2 followed by addition of $NaIO_4$ (9.89 g / 46.28 mmol) and then 4 mL of aqueous saturated $NaHCO_3$. The resulting mixture was mechanically stirred at room temperature for 3 hours before the addition of 20 g of $MgSO_4$, then stirred at room temperature for another 15 minutes. The solution was then filtered, and the solvent removed under reduced pressure to give the crude aldehyde that was dissolved into 100 mL of 9 : 1 MeOH : H_2O which also contained 14.58 g / 173.55 mmol of $NaHCO_3$. Br_2 (4.8 mL / 93.78 mmol) was then added dropwise to the solution and the resulting orange mixture was stirred overnight at room temperature. Saturated aqueous sodium bisulfite was then added dropwise to the solution until the orange color disappeared. Methanol was removed under reduced pressure, and the remaining aqueous phase was extracted with CH_2Cl_2 to yield the crude product which was purified by flash chromatography to give the pure product as a colorless oil (4.81 g, 65% yield from **94**).[65] ^{1}H NMR: ($CDCl_3$) δ 1.42 (s, 3H), 1.45 (s, 3H), 3.80 (s, 3H), 4.12 (m, 1H), 4.23 (m, 1H), 4.61 (m, 1H). ^{13}C NMR: ($CDCl_3$) δ 25.66, 26.00, 52.52, 67.40, 74.20, 111.50, 177.78.



95

A round bottom flask equipped with an addition funnel was flame dried while purging with Argon before the addition of 25 mL of THF and then cooled to -10^o C. *n*-butyllithium (13.34 mL

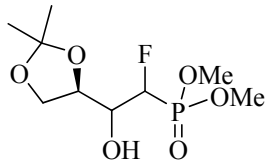
/ 33.34 mmol from a 2.5 M solution) was then added and the mixture was cooled to -78° C. Dimethyl methylphosphonate **96** (3.58 mL / 31.75 mmol) in 7 mL of THF was then added dropwise to the solution of *n*-BuLi at -78° C and the resulting white slurry was stirred at -78° C for 30 minutes. The slurry was then transferred via cannula to a separate flask containing methyl ester **95** (4.37 mL / 30.16 mmol) in 15 mL of THF at -78° C. The resulting solution was stirred at -78° C for one hour before allowing to warm to room temperature overnight. Saturated ammonium chloride (10mL) was added dropwise at room temperature to quench the reaction and THF was removed under reduced pressure. The resulting aqueous layer was extracted with EtOAc (3 x 20 mL), the combined organic layers were dried with $MgSO_4$ and the solvent removed under reduced pressure to give the crude product as an oil which was purified by flash chromatography (3:1 Hex : EtOAc to 3 : 1 EtOAc : Hex) to give the pure product as a colorless oil. (6.89 g, 86% yield).[83] ^{1}H NMR: ($CDCl_3$) δ 1.37 (s, 3H), 1.46 s (3H), 3.16 (m, 1H), 3.49 (m, 1H), 3.77 (d, 2H, J = 3.6 Hz), 4.35 (m, 4H), 4.55 (m, 1H). ^{13}C NMR: ($CDCl_3$) δ 25.14, 26.21, 35.81, 37.55, 53.31 (m), 66.18, 80.32, 111.43, 202.39. ^{31}P NMR: ($CDCl_3$) δ 23.26.



88

A round bottom flask equipped with an addition funnel was charged with NaH (0.15 g / 3.7 mmol of a 30% dispersion) and the NaH was washed two times with 5 mL of pentane before addition of 15 mL of THF. The resulting solution was cooled to 0° C and phosphonate **97** (850 μL / 3.7 mmol) dissolved in 5 mL THF was added dropwise to the flask at 0° C. The resulting solution was stirred at 0° C for 30 minutes and room temperature for one hour before recooling to 0° C. This solution was then transferred via cannula to a suspension of Selectfluor (1.31 g /

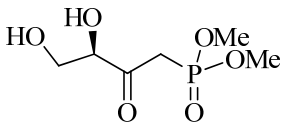
3.7 mmol) in dry CH_3CN at 0° C and the resulting solution stirred at 0° C for one hour before warming to room temperature overnight. The reaction was quenched with saturated ammonium chloride (7 mL), THF and acetonitrile were removed under reduced pressure and the resulting aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organics were dried with $MgSO_4$ and the solvent removed under reduced pressure to give the crude product as an oil. Purification via flash chromatography (5 : 1 Hex : EtOAc to 1:1 Hex : EtOAc) gave the pure product as a slightly yellow oil. ^{1}H NMR: ($CDCl_3$) δ 1.46 (s, 3H), 1.51 (s, 3H), 3.80 (m, 4H), 4.25 (m, 2H), 4.9 (m, 1H), 5.4 (dd, *J* = 47.21, 60.39 Hz), 5.75 (dd, *J* = 47.5, 47.5 Hz). ^{19}F NMR: ($CDCl_3$) δ -215.5 (dd, *J* = 70.24, 70.24 Hz), -216.0 (dd, *J* = 69.03, 70.24 Hz).



98

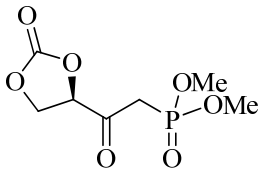
A round bottom flask containing $NaBH_4$ (0.017 g / 0.44 mmol) in MeOH (4 mL) was cooled to 0° C before the addition of ketone **88** (0.12 g / 0.44 mmol). The solution was stirred at 0° C for one hour and at room temperature for 4 hours before the reaction was quenched with saturated aqueous ammonium chloride (1 mL). MeOH was then removed under reduced pressure and the resulting aqueous layer extracted with CH_2Cl_2 (3 x 7 mL). The combined organics were dried with $MgSO_4$, the solvent evaporated under reduced pressure and the crude oil purified by flash chromatography (1 : 1 Hexanes : EtOAc to 4 : 1 Hexanes : EtOAc) to give the product as a colorless oil. ^{1}H NMR: $CDCl_3$ δ 1.38 (m, 6H), 3.80 (m, 10H), 4.70 (m,1H). ^{13}C NMR: ($CDCl_3$) δ 25.16, 25.20, 25.23, 26.17, 26.41, 26.70, 26.91, 65.42, 65.80, 66.36, 67.00, 68.71, 69.01, 69.74, 69.98, 69.74, 69.98, 70.87, 71.10, 71.87, 72.13, 73.73, 73.79, 73.88, 74.54, 74.69, 75.26, 75.46, 75.15, 75.61, 75.66, 84.94, 86.14, 87.18, 87.41, 88.37, 88.64, 89.65, 90.87.

^{31}P NMR: ($CDCl_3$) δ 18.89, 19.27, 19.50, 19.90, 20.18, 20.32, 20.90. ^{19}F NMR: ($CDCl_3$) δ -213.4, dddd (J = 46.02 Hz), -215.4, dddd (J = 44.81, 46.02, 46.02, 44.81 Hz), -223.4 dddd (J = 46.02Hz), -230.7 dddd (J = 44.81, 46.24, 44.81, 44.81 Hz).



101

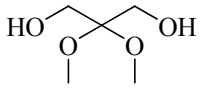
Acetonide protected phosphonate **97** (0.41 g / 1.63 mmol) was dissolved in 7 mL of MeOH at room temperature before the addition of 0.6 g of Dowex 50WX8-100 resin at room temperature. The resin was then filtered and washed multiple times with acetone to remove the diol product. Acetone and methanol were removed under reduced pressure and the resulting oil / water mixture was azeotroped several times with CH_2Cl_2 to remove the residual water. The resulting oil was then purified by flash chromatography (EtOAc) to give the pure product as a slightly yellow oil (72 % / 0.25 g). ^{1}H NMR: ($CDCl_3$) δ 3.35 (d, 2H J = 3.8 Hz), 3.80, (m, 6H), 3.92 (m, 1H), 4.05 (m, 1H), 4.27 (m, 1H). ^{13}C NMR: ($CDCl_3$) δ 36.00, 37.76, 48.31, 48.61, 50.48, 62.68, 77.89. ^{31}P NMR: ($CDCl_3$) δ 23.74.



102

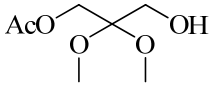
A flask was charged with diol **101** (0.44 g / 2.09 mmol) and pyridine (0.51 mL / 6.27 mmol) in CH_2Cl_2 (15 mL) and then cooled to -40^o C under Argon. Triphosgene (0.62 g / 2.09 mmol) dissolved in CH_2Cl_2 (5 mL) was then added dropwise at the same temperature. The solution was allowed to warm slowly to room temperature over two hours and then stirred for an additional two hours at room temperature. The resulting solution was washed with 10 % HCl

(10 mL), saturated $NaHCO_3$ (10 mL) and brine (10 mL), dried with $MgSO_4$, and the solvent removed under reduced pressure to give the crude product as an oil. The crude product was purified by flash chromatography (2 : 1 Hexanes : EtOAc) to give the pure product as a yellow oil. ^1H NMR: ($CDCl_3$) δ 3.42 (m, 2H), 3.82 (m, 6H), 4.64 (m 2H), 5.32 (m, 1H). ^{31}P NMR: ($CDCl_3$) δ 21.18.



110

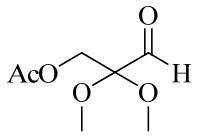
Dihydroxyacetone dimer (7.5 g / 41.6 mmol), trimethylorthoformate (9 mL / 82.2 mmol), and p-TsOH (0.03 g / 0.16 mmol) and anhydrous MeOH (100 mL) were combined in a flask and stirred under Argon overnight at room temperature. Amberlyst A26 resin (0.28 g) was then added at room temperature and the solution was stirred at room temperature for 15 minutes. The solution was then filtered and the solvent was removed under reduced pressure to give a white solid (5.37 g / 95 % yield) which was pure enough for further use. The solid could be purified by flash chromatography (1 : 1 Hexanes : EtOAc) if desired.[72] ^1H NMR: ($CDCl_3$) δ 3.25 (s, 6H), 3.70 (s, 4H). MS: 105 (M – 31, 4.5%), 77 (100%), 55 (35.4%).



111

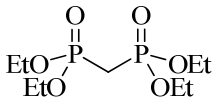
Protected diol **110** (3.75 g / 27.6 mmol), *Pseudomonas fluorescens* lipase (0.33 g), vinyl acetate (40 mL) and diisopropyl ether (20 mL) were stirred under Argon overnight at room temperature. The lipase was removed by filtration over Celite and the solvents removed under reduced pressure. The resulting oil was purified by flash chromatography(5 : 1 Hexanes : EtOAc to 3 : 1 Hexanes : EtOAc) to give the pure compound as a colorless oil (2.95 g / 60% yield).[72] ^1H NMR: ($CDCl_3$) δ 2.18 (s, 3H), 3.25 (s, 6H), 3.60 (s, 2H), 4.20 (s, 2H). ^{13}C NMR: ($CDCl_3$) δ

20.47, 48.06, 59.80, 99.81, 170.86. FT-IR: (neat) 3463.7, 295.1, 1711.7. MS: 147 (100%), 129 (1.7%), 105 (62.9%), 87 (28.5%), 73 (35.2%).



112

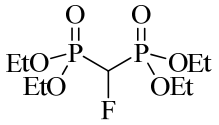
An oven dried round bottom flask was charged with DMSO (257 μL / 3.54 mmol) in 15 mL of dry CH_2Cl_2 and cooled to -60° C under Argon. Oxalyl chloride (280 μL / 3.27 mmol) was then added dropwise at -60° C and the resulting solution stirred at this temperature for 10 minutes. Acetylated alcohol **103** (450 μL / 2.83 mmol) was then added dropwise to the solution at -60° C followed by stirring at -60° C for 20 minutes before quenching via dropwise addition of NEt_3 (1.3 mL) at -60° C. The solution was stirred at -60° C for 15 minutes before allowing to warm to room temperature over 45 minutes. Solvents were then removed under reduced pressure and the resulting residue dissolved into half saturated $NaHCO_3$ (6 mL) and extraction of the aqueous layer with ether (3 x 10 mL). The combined organics were washed with brine (5 mL), dried with $MgSO_4$, and the solvents removed under reduced pressure to give the crude compound (0.42 g / 85% crude yield) as a slightly yellow oil which was used without further purification. ^{1}H NMR: ($CDCl_3$) δ 2.05 (s, 3H), 3.30 (s, 6H), 4.25 (s, 2H), 9.45 (s, 1H). ^{13}C NMR: ($CDCl_3$) δ 20.80, 50.16, 61.11, 66.59, 170.1, 204.12. FT-IR: (neat) 2950.02, 1750.32, 1172.08. MS: 147 (100%), 133 (0.9%), 115 (5.6%), 103 (56.9%), 73 (31.3%).



107

A flame dried round bottom flask was charged with diisopropylamine (8.85 mL / 63.14 mmol) and THF (15 mL), then cooled to -78° C before the dropwise addition of *n*-butyllithium

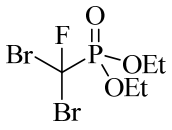
(60.2 mL / 66.2 mmol) at the same temperature. The anion was allowed to stir at -78° C for 30 minutes before the dropwise addition of diethylmethyl phosphonate (4.5 mL / 30.8 mmol) in 5 mL of THF at -78° C. The resulting solution was stirred at -78° C for 30 minutes before the dropwise addition of diethyl chlorophosphate (4.7 mL / 32.3 mmol) in 5 mL of THF. The resulting solution was stirred for 30 minutes at -78° C before slowly allowing to warm to -35° C over a period of 2 hours. The solution was then cooled to -50° C and quenched by the dropwise addition of 3M HCl (22 mL). The resulting viscous mixture was allowed to warm to room temperature and THF was removed by rotary evaporation. The resulting aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organics were dried with $MgSO_4$ and the solvent removed under reduced pressure. The resulting oil was dissolved into 60 mL of anhydrous ether and the triethylammonium salts removed by filtration. The ether was removed to give the crude product as a yellow oil. The product was purified by vacuum distillation (165° C bath temperature / 125° C apparatus temperature / 0.05 mm Hg) to give the pure compound as a slightly yellow oil (8.0 g / 90% yield).[70] ^{1}H NMR: ($CDCl_3$) δ 1.30 (t, 12H, J = 7.0 Hz), 2.4 (t, 2H J = 21.0 Hz), 4.05 (m, 8H). ^{13}C NMR: ($CDCl_3$) δ 16.42, 23.61, 25.42, 27.24, 62.72. ^{31}P NMR: ($CDCl_3$) δ 20.51. IR: (neat) 2985.30, 1480.10, 1024.76. MS: 288 (m/z, 10.2%), 261 (40.8%), 205 (2.0%), 177 (0.6%), 159 (100%), 125 (65.7%).



108

Potassium hydride (0.29 g / 2.21 mmol of a 30% dispersion in mineral oil) was washed twice with pentane before the addition of 7 mL of THF. The resulting suspension was then cooled to 0° C before the dropwise addition of bisphosphonate **107** (0.5 mL / 2.01 mmol) in 5 mL of THF. The resulting solution was stirred for 30 minutes at 0° C and one hour at room

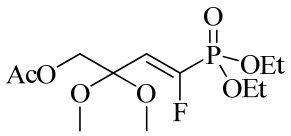
temperature before recooling to 0° C. The anion was then transferred via cannula to a solution of Selectfluor (0.71 g / 2.01 mmol) in CH_3CN (20 mL). The resulting solution was then allowed to warm to room temperature overnight before the addition of saturated NH_4Cl (5 mL). THF and acetonitrile were then removed under reduced pressure and the remaining aqueous layer was extracted with ether (3 x 15 mL). The combined organics were dried with $MgSO_4$ and the solvent removed under reduced pressure to give the crude compound as an oil. Purification by flash chromatography (4.5 : 1 Hexanes : EtOAc to 2: 1 EtOAc : Hex) gave the pure compound as a colorless oil (1.62 g / 50% yield).[82,84] CAUTION: If this procedure is used the purification requires a much longer time than for standard organic compounds. ^{1}H NMR: ($CDCl_3$) δ 1.20 (t, 12H J = 7.1 Hz), 4.05 (m, 8H), 4.82 (dt, 1H J = 13.4, 45.9 Hz). ^{13}C NMR: ($CDCl_3$) δ 16.15, 64.81 (d, J = 30.72 Hz), 84.05 (dt J = 156.1, 312.7 Hz). ^{31}P NMR: ($CDCl_3$) δ 11.05 (d, J = 64.09 Hz). ^{19}F NMR: ($CDCl_3$) δ 312.6 (dt J = 62.3, 45.9 Hz). FT-IR: (neat) 2984.95, 1260.04, 1026.07. MS: 306 (m/z, 7.1%), 279 (41.4%), 223 (61.4%), 194 (80.8%), 177 (100%), 143 (50.6%).



40

Tribromofluoromethane (1.35 mL / 13.8 mmol), triethyl phosphite (2.36 mL / 13.8 mmol) and THF (12 mL) were combined in a round bottom flask and heated to 50° C and stirred overnight at this temperature. The reaction was then cooled, the solvent removed under reduced pressure and the resulting oil purified by flash chromatography (12 : 1 Hexanes : EtOAc) to give the pure product as a slightly yellow oil (4.30 g / 95% yield). ^{1}H NMR: ($CDCl_3$) δ 1.42 (t, 6H J = 7.2 Hz), 4.39 (m, 4H). ^{13}C NMR: ($CDCl_3$) δ 16.60 (d, J = 10 Hz), 66.99 (d, J = 7.0 Hz). ^{31}P NMR: ($CDCl_3$) δ 2.55 (d, J = 76.9 Hz). ^{19}F NMR: ($CDCl_3$) δ 76.50 (d, J = 76.8 Hz). IR: (neat)

2984.95, 1274.84, 1018.98. MS: 327 (m/z, 0.1%), 299 (1.1%), 247 (2.6%), 191 (9.2%), 137 (100%), 109 (90.8%).

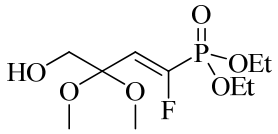


113

From purified fluorinated bisphosphonate **108**: An oven dried round bottom flask was charged with fluorinated bisphophonate **108** (100 μL / 0.38 mmol) in 6 mL of THF and cooled to -78° C before the dropwise addition of *n*-BuLi (345 μL / 0.38 mmol from a 1.1 M solution). The resulting solution was stirred at -78° C for 10 minutes before the addition of crude aldehyde **112** (84 μL / 0.52 mmol) at the same temperature. The solution was then allowed to stir at -78° C for 1.5 hour before slowly allowing to warm to room temperature overnight. Water (3 mL) was then added, THF was removed under reduced pressure and the resulting aqueous solution was extracted with ether (3 x 8 mL). The combined organic layers were dried with $MgSO_4$ and the solvent removed under reduced pressure to give the crude product as a yellow oil which was purified by flash chromatography (3 : 1 Hexanes : EtOAc to 1.5 : 1 Hexanes : EtOAc) to give the pure product as a slightly yellow oil (0.088 g / 70% yield). ^{1}H NMR: ($CDCl_3$) δ 1.40 (t, 6H, J = 7.0 Hz), 2.05 (s, 3H), 3.25 (s, 6H), 4.15 (m, 4H), 4.25 (s, 2H), 5.85 (dd, J = 9.24, 41.91 Hz, 1H). ^{13}C NMR: ($CDCl_3$) 16.14, 16.22, 20.66, 49.05, 62.80, 63.37 (d, J = 5.7 Hz), 99.65 (d, J = 14.6 Hz), 122.3 (d, J = 27.5 Hz), 152.1 (dd, J = 231.6, 288.6 Hz), 170.0. ^{31P} NMR: ($CDCl_3$) δ 4.7 (d, J = 100.72). ^{19}F NMR: δ -120.4 (dd, J = 41.52, 99.65). FT-IR: (neat) 2985.09, 1751.85, 1020.20. MS: 297 (M – 31, 7.1%), 277 (3.1%), 235 (48%), 207 (15.5%), 99 (68.8%).

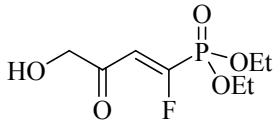
From α-dibromofluorophosphonate **40**: A flame dried round bottom flask was charged with THF (7 mL) and *n*-BuLi (325 μL / 0.52 mmol from a 1.6 M solution) and cooled to -78° C

followed by dropwise addition of phosphonate **40** (100 μL / 0.52 mmol) at -78° C (the solution turned red / brown at this point). The resulting solution was stirred at -78° C for one hour before dropwise addition of aldehyde **112** at the same temperature. The solution was then stirred at -78° C for one hour before allowing to warm to room temperature overnight. Water (5 mL) was then added, THF was removed under reduced pressure and the aqueous layer extracted with ether (3 x 10 mL). The combined organics were dried with $MgSO_4$ and the solvent removed under reduced pressure to give the crude product as a brown oil which was purified by flash chromatography using the same solvent system as listed above to give the product as a slightly yellow oil (55.4 mg / 65% yield). Spectral data were also the same as listed above.



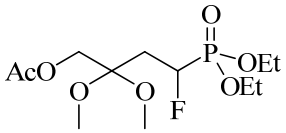
114

A round bottom flask containing acetylated alcohol **113** (0.65 g / 1.98 mmol) and Amberlys A26 resin in 8 mL MeOH was stirred overnight at room temperature. The resin was removed by filtration then washed with acetone. The resulting solution was concentrated under reduced pressure and the crude oil purified by flash chromatography (2 : 1 Hexanes : EtOAc to 1.5 : 1 EtOAc to Hexanes) to give the pure product as a pale yellow oil (0.38 g / 75% yield). ^{1}H NMR: ($CDCl_3$) δ 1.40 (t, 6 H, J = 7.1 Hz), 3.15 (s, 6H), 3.78 (s, 2H), 4.20 (m, 4H), 5.85 (dd, J = 9.4, 42.51 Hz, 1H). ^{13}C NMR: ($CDCl_3$) δ 18.36, 49.40, 63.37, 63.66 (d, J = 5.4 Hz), 101.47(d, J = 14.3 Hz), 123.12 (d, J = 27.5 Hz), 152.34 (dd, J = 287.1, 233.0 Hz). ^{31}P NMR ($CDCl_3$) δ 4.8 (d, J = 102.54). ^{19}F NMR ($CDCl_3$) δ -120.85 (dd, J = 41.44, 101.73 Hz). IR (neat) v 3418.12, 2985.48, 1019.28. MS 255 (M – 31, 66.4%), 235 (46.3%), 207 (14.0%), 179 (23.2%), 167 (17.3%), 117 (30.2%), 99 (100%).



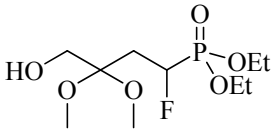
104a

A round bottom flask containing alcohol **113** (0.55 g / 1.93 mmol) and Montmorillonite clay (1 g) in 10 mL of CH_2Cl_2 was stirred at room temperature overnight. The solution was then filtered over Celite, the solvent was removed under reduced pressure and the resulting oil purified by flash chromatography (1 : 1 Hexanes : EtOAc to 3 : 1 EtOAc to Hexanes) to give the pure product as a slightly yellow oil (0.26 g / 65% yield).[85] ^{1}H NMR: ($CDCl_3$) δ 1.40 (t, 6H, J = 7.0 Hz), 4.20 (m, 4H), 4.58 (s, 2H), 6.35 (dd, J = 8.2, 42.51 Hz). ^{13}C NMR: ($CDCl_3$) 16.54, 64.55 (d, J = 5.8 Hz), 69.85, 116.85 (d, J = 25.9 Hz), 161.26 (dd, J = 307.2 Hz, 225.6 Hz), 196.10. ^{31}P NMR: ($CDCl_3$) δ 2.70 (d, J = 100.11 Hz). ^{19}F NMR: ($CDCl_3$) δ -101.6 (dd, J = 43.6, 101.73 Hz). FT-IR: (neat) 3418, 2897, 1699, 1642, 1019.



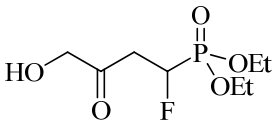
116

A round bottom flask was charged with fluorinated alkene **113** (0.50 g / 1.52 mmol), EtOAc (8 mL) and a spatula tip of PdOH / C before hydrogenation overnight at 1 atm at room temperature. The suspension was then filtered over Celite and the solvents removed under reduced pressure to give the crude product as a slightly yellow oil. The crude product was purified by flash chromatography (6 : 1 Hex : EtOAc to 2 : 1 Hex : EtOAc) to give the pure product as a slightly yellow oil (0.15 g / 30% yield). ^{1}H NMR: ($CDCl_3$) δ 1.32 (t, 6H, J = 7.2 Hz), 2.05 (s, 3H), 2.29 (m , 2H), 3.05 (s, 6H), 3.40 (s, 2H), 4.10 (m , 4H), 4.87 (m, 1H). ^{31}P NMR: ($CDCl_3$) δ 19.02 (d, J = 75.69 Hz). ^{19}F NMR: ($CDCl_3$) δ -208.3 (m).



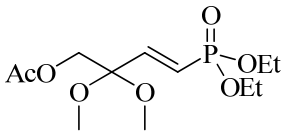
117

A round bottom flask was charged with reduced phosphonate **116** (0.15 g / 0.45 mmol), Dowex A26 resin (0.10 g) and Methanol (6 mL) before stirring overnight at room temperature. The solution was then filtered, and the filtrered resin washed several times with acetone. Solvents were then removed under reduced pressure to give the crude compound as a slightly yellow oil. The crude product was purified by flash chromatography (2 : 1 Hex : EtOAc to 2 : 1 EtOAc : Hex) to give the pure product as a slightly yellow oil (0.097 g / 75% yield). 1H NMR: ($CDCl_3$) δ 1.40 (t, 6H, *J* = 7.1 Hz), 2.20 (m, 2H), 3.10 (s, 3H), 3.40 (s, 2H), 4.10 (m, 4H), 4.79 (m, 1H).



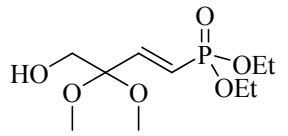
115
(crude)

A round bottom flask was charged with alcohol **117** (0.097 g / 0.34 mmol), montmorillonite clay (0.10 g) and CH_2Cl_2 (5 mL) before stirring at room temperature overnight. The suspension was then filtered over Celite and the solvents removed under reduced pressure to give the crude product as a yellow oil which contained the desired α-fluorophosphonate as the major product plus defluorinated vinyl phosphonate **119** as an inseparable byproduct (crude yield 80 % / 0.065 g). ^{1}H NMR: ($CDCl_3$) δ 1.25 (m, 6H), 3.05 (m, 2H), 4.12 (m, 4H), 4.20 (s, 2H), 5.31 (m, 1H), 6.90 (m, 2H, this signal is from the defluorinated vinyl phosphonate side product). ^{31}P NMR: ($CDCl_3$) δ 17.35 (d, *J* = 73.25 Hz), 15.35 (s, this signal is from the defluorinated vinyl phosphonate side product). ^{19}F NMR: ($CDCl_3$) δ 208.13 (m).



120

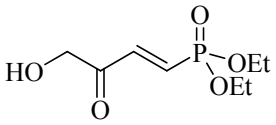
An oven dried round bottom flask was charged with bisphosphonate **107** (646 μL / 2.60 mmol) in THF (10 mL) and cooled to -78° C under Argon. *n*-Butyllithium (1.04 mL from a 2.5 M solution in hexanes) was then added dropwise at -78° C and the resulting solution stirred at the same temperature for 1 hour. Aldehyde **112** (569 μL / 3.56 mmol) was then added dropwise at -78° C and the resulting solution stirred at the same temperature before allowing to warm overnight. Water (3 mL) was then added, and THF was removed under reduced pressure. The remaining aqueous layer was extracted with ether (3 x 10 mL), the combined organics were washed with brine (4 mL), dried with $MgSO_4$ and the solvent removed under reduced pressure to give the crude product as a slightly yellow oil. The crude product was purified by flash chromatography (4 : 1 Hex : EtOAc) to give the pure product as a colorless oil (0.67 g / 82% yield). ^{1}H NMR: ($CDCl_3$) δ 1.30 (t, 6H, *J* = 7.0 Hz), 2.05 (s, 3H), 3.20 (s, 6H), 4.05 (m, 4H), 4.08 (s, 2H), 6.15 (dd, 1H, *J* = 20.3, 17.5 Hz), 6.50 (dd, 1H, *J* = 22.53, 17.3 Hz). ^{31}P NMR: ($CDCl_3$) δ 18.18.



121

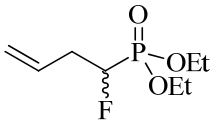
A round bottom flask was charged with vinyl phosphonate **120** (0.67 g / 2.13 mmol), A26 resin (0.70 g) and methanol (8 mL) and then stirred at room temperature overnight. The product was then filtered, the resin was washed several times with acetone and the solvents evaporated to give the crude product as a slightly yellow oil. The crude product was purified by flash

chromatography (1 : 1 Hex : EtOAc) to give the pure product as a slightly yellow oil (0.44 g / 78 % yield). ^{1}H NMR: ($CDCl_3$) δ 1.21 (t, 6H *J* = 7.1 Hz), 3.05 (s, 6H), 3.50 (s, 2H), 3.98 (m, 4H), 6.25 (m, 2H). ^{31}P NMR: ($CDCl_3$) δ 18.71.



119

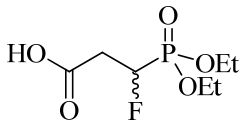
A round bottom flask was charged with alcohol **121** (0.44 g / 1.66 mmol), montmorillonite clay (0.55 g) and CH_2Cl_2 (7 mL) before stirring overnight at room temperature. The suspension was then filtered over Celite and the solvent removed under reduced pressure to give the crude product as a yellow oil. The crude product was purified by flash chromatography (1 : 1 Hex : EtOAc) to give the pure product as a slightly yellow oil (0.33 g / 90% yield). ^{1}H NMR: ($CDCl_3$) δ 1.25 (t, 6H *J* = 7.1 Hz), 4.05 (m, 4H), 4.42 (s, 2H), 6.80 (m, 2H). ^{31}P NMR: ($CDCl_3$) δ 15.44 (s).



122

A three neck round bottom flask equipped with a pressure equalizing dropping funnel was flame dried while purging with Argon before charging the flask with *n*-Butyllithium (6.9 mL / 17.1 mmol from a 2.5 M solution in hexanes) in THF (20 mL). The dropping funnel was charged with a mixture of dibromofluorophosphonate **40** (1.5 mL / 7.83 mmol) and trimethylsilyl chloride (993 μL / 7.83 mmol) in THF (10 mL). The flask was then cooled to -78^{o} C and the contents of the funnel were added dropwise at this temperature. The resulting brown solution was stirred at -78^{o} C for 10 minutes before the dropwise addition of allyl iodide (927 μL / 10.2

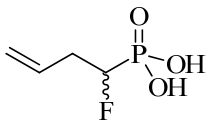
mmol) in THF (7 mL) at the same temperature. The resulting solution was stirred at -78° C for 45 minutes, then allowed to warm to 0° C on an ice bath. A solution of lithium ethoxide (prepared by adding 0.3 g of lithium wire to ethanol at room temperature) was then added dropwise at 0° C and the resulting solution was allowed to stir at the same temperature for 1 hour before pouring into 15 mL of 2 M HCl. THF was removed under reduced pressure, and the remaining aqueous solution was extracted with ether (3 x 15 mL). The combined organic phases were washed with a freshly prepared solution of saturated sodium bisulfite (3 x 7 mL) and brine (1 x 7 mL), dried with $MgSO_4$ and the solvent removed under reduced pressure to give the crude product as a dark yellow oil. The crude product was purified by flash chromatography (12 : 1 Hex : EtOAc to 8 : 1 Hex : EtOAc) to give the pure product as a slightly yellow oil (1.40 g / 85% yield).[86] ^{1}H NMR: ($CDCl_3$) δ 1.25 (t, 6H J = 7.05 Hz), 2.6 (m, 2H), 4.10 (m, 4H), 4.72 (m, 1H), 5.14 (m, 2H). ^{13}C NMR: δ 15.42, 34.05, 62.31 (m), 67.46, 67.52, 112.48, 131.12. ^{31}P NMR: ($CDCl_3$) δ 17.8 (d, J = 74.26 Hz). ^{19}F NMR: ($CDCl_3$) δ -200.67 (m).



123

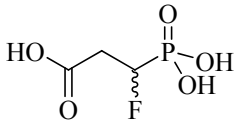
A round bottom flask was charged with $NaHCO_3$ (0.37 g / 4.5 mmol) followed by addition of a 0.1 M aqueous solution of $RuCl_3$ (139 μL) then CH_3CN (8.4 mL) and EtOAc (8.4 mL). Oxone was then added in one portion to the brown suspension to give a bright yellow suspension (gas was evolved at this point). The reaction mixture was then cooled to 0° C in an ice bath and unsaturated phosphonate **122** (0.30 g / 1.39 mmol) was added in one portion. The mixture was stirred at 0° C for one hour before dilution with EtOAc (20 mL), the mixture was then filtered over Celite followed by washing of the solution with brine (1 x 5 mL). The organic phase was dried with $MgSO_4$ and the solvent removed under reduced pressure to give the crude product as a

yellow oil. Purification by flash chromatography (3 : 1 EtOAc : Hex) gave the product as a colorless oil (0.11 g / 34% yield). ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 6H, J = 7.1 Hz), 2.92 (m, 2H), 4.05 (m, 4H), 5.25 (m, 1H). ^{31}P NMR: ($CDCl_3$) δ 17.82 (d, J = 74.47 Hz). ^{19}F NMR: ($CDCl_3$) δ -203.45 (m).



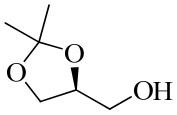
125

A round bottom flask was charged with unsaturated phosphonate **122** (0.10 g / 0.48 mmol) and then placed under vacuum for four hours. The flask was then sealed with a rubber septa and placed under Argon and TMSBr (218 μL / 1.61 mmol) was added dropwise to the neat phosphonate at room temperature. The rubber septa was removed and quickly replaced with a yellow Caplugs stopper and the reaction was stirred at room temperature overnight. Volatile materials were then removed (first on the rotovap, then on high vacuum for four hours) and water (1.5 mL) was added to the resulting oil and the solution stirred overnight at room temperature. (The solution instantly became turbid before slowly clearing overnight). Water was then removed by freeze drying to yield the phosphonic acid product as a colorless, viscous oil which was used without further purification in the next step (0.07 g / 95% yield). ^{1}H NMR: (D_2O) δ 2.25 (m, 2H), 4.60 (m, 1H), 4.91 (m, 2H), 5.62 (m, 1H). ^{13}C NMR: δ (D_2O) 40.15, 62.45 (m), 112.76, 132.04. ^{31}P NMR: (D_2O) δ 16.34 (d, J = 74.49 Hz). ^{19}F NMR: (D_2O) δ -200.48 (m).



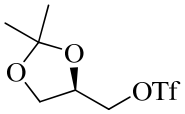
126

This compound was prepared in the same way as **123** in 25%yield. ^{1}H NMR: (D_2O) δ 2.45 (m, 2H), 4.62 (m, 1H). ^{13}C NMR: δ (D_2O) 39.90 (d, J = 21.7 Hz), 91.05 (dd, J = 169.7, 153.1 Hz), 180.11. ^{31}P NMR: (D_2O) δ 14.55 (d, J = 70.11 Hz). ^{19}F NMR: (D_2O) δ -204.75 (m).



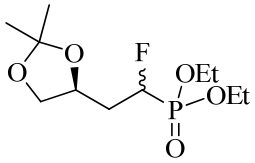
127

Protected D-mannitol was cleaved as described for the synthesis of ester **95**. The resulting aldehyde (5.16 g / 39.7 mmol) was dissolved in EtOH (45 mL) and the solution cooled to 0^{o} C before the slow addition of $NaBH_4$ over a period of five minutes. The solution was then allowed to warm to room temperature and stirred at room temperature for two hours. The reaction was then quenched by the addition of 3 M HCl until the pH was neutral. EtOH was then removed under reduced pressure and the aqueous phase was extracted with CH_2Cl_2 (4 x 40 mL). The combined organic phases were dried with $MgSO_4$ and the solvent removed under reduced pressure to give the crude product as a colorless oil. The crude product was purified by flash chromatography (10 : 1 Hex : EtOAc) to give the pure product as a colorless oil (4.0 g / 76% yield).[87] ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3H) 1.42 (s, 3H), 3.58 (m, 1H), 3.74 (m, 1H), 3.80 (m, 1H), 4.05 (m, 1H), 4.24 (m, 1H). ^{13}C NMR δ ($CDCl_3$) 25.6, 26.8, 62.5, 65.0, 77.1, 108.4.



128

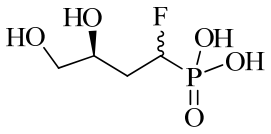
A solution of alcohol **127** (750μL / 6.30 mmol) and pyridine (627 μL / 7.75 mmol) in CH_2Cl_2 (15 mL) was cooled to -60° C before the addition of triflic anhydride at the same temperature. The resulting solution was stirred at -60° C for 30 minutes before allowing to warm to room temperature over one hour. The organic layer was washed with brine (1 x 6 mL), dried with $MgSO_4$, and concentrated under reduced pressure to give a dark brown / purple oil which was used immediately in the next step without further purification.[77]



129

A round bottom flask equipped with a pressure equalizing dropping funnel was flame dried while purging with Argon before charging with *n*-butyllithium (2.71 mL / 10.6 mmol of a 2.0 M solution in hexanes) in THF (15 mL). Dibromofluorophosphonate **40** (469 μL / 2.45 mmol) and TMSCl (2.45 mmol / 310 μL) in THF (10 mL) were added to the dropping funnel. The flask was cooled to -78° C before the dropwise addition of the contents of the dropping funnel at the same temperature. The resulting dark yellow / brown solution was stirred at -78° C for 15 minutes before the addition of triflate **128** (0.65 g / 2.45 mmol) in THF (8 mL) at the same temperature. This solution was stirred at -78° C for one hour before warming to 0° C followed by the dropwise addition of a solution of lithium ethoxide (prepared by adding 0.2 g of lithium wire to 12 mL of ethanol) and the resulting solution allowed to stir at 0° C for one hour before the solution was poured into 7 mL of saturated NH_4Cl. THF was removed under reduced pressure and the aqueous layer extracted with EtOAc (3 x 20 mL). The combined organics were dried with $MgSO_4$ and the solvent removed under reduced pressure to give the crude product which was purified by flash chromatography (8 : 1 Hex : EtOAc to 2 : 1 Hex : EtOAc) to give the pure

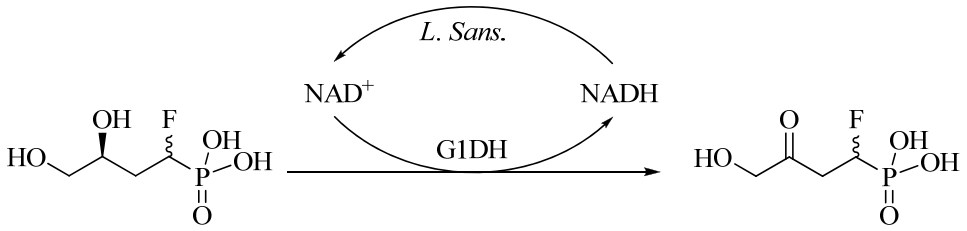
product as a slightly yellow oil (0.49 g / 70%).[46] ^{1}H NMR: ($CDCl_3$) δ 1.25 (m, 12H), 2.10 (m, 2H), 3.48 (m, 1H), 4.10 (m, 6H), 4.80 (m, 1H). ^{13}C NMR: δ ($CDCl_3$) 16.5 (d, *J* = 3.2 Hz), 25.6, 26.8 27.0, 33.8 (d, *J* = 19.75 Hz), 34.95, 62.9, 63.1 (d, *J* = 6.60 Hz), 63.5 (m), 68.8, 69.4, 71.7 (d, *J* = 14.89 Hz), 72.5 (d, *J* = 11.74 Hz), 83.8 (d, *J* = 17.75 Hz), 86.1 (dd *J* = 7.73, 18.32 Hz), 88.4 (d, *J* = 17.46 Hz), 109.26. ^{31}P NMR: ($CDCl_3$) δ 18.67 (d *J* = 74.07 Hz), 18.35 (d *J* = 75.30 Hz). ^{19}F NMR ($CDCl_3$): δ -207.9 (m), -212.3 (m).



63

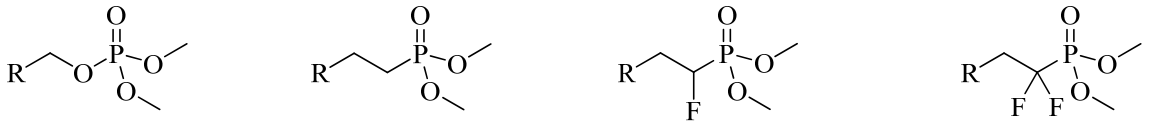
A round bottom flask charged with protected phosphonate **129** (100 μL / 0.39 mmol) was dried under high vacuum for four hours before sealing with a rubber septa followed by the dropwise addition of TMSBr (302 μL / 2.30 mmol) to the neat compound at room temperature. The rubber septa was immediately replaced with a yellow caplugs stopper, and the resulting solution stirred at room temperature for four hours. Volatile materials were then removed; first on the rotovap and then on high vacuum for three hours. Water (1 mL) was then added, and the resulting solution was stirred overnight at room temperature. The resulting solution was then loaded onto a column of Dowex IX-8 resin (HCO_3^- form, prepared by washing the resin sequentially with di H_2O, 1 M $NaHCO_3$ and diH_2O until the pH was neutral) and eluted with 100 mM NH_4HCO_3. The eluant was then freeze dried to give the ammonium salt as a white powder. The free phosphonic acid was obtained by several cycles of freeze drying. ^{1}H NMR: (D_2O) δ 1.65 (m, 2H), 3.25 (m, 2H), 3.65 (m, 1H), 4.65 (m, 1H). ^{13}C NMR: (D_2O) 34.35 (d, *J* = 19.5 Hz), 64.74, 65.60, 68.10 (dd, *J* = 2.9, 11.2 Hz), 69.80 (dd, *J* = 2.3, 11.5 Hz), 89.45 (dd, *J* =

169.8, 133.45 Hz), 91.23 (dd, *J* = 169.5, 154.3 Hz). ^{31}P NMR: (D_2O) δ 16.8 (*d*, J = 71.4 Hz), 17.0 (*d*, J = 70.11 Hz). ^{19}F NMR: (D_2O) δ -203.75 (m), -207.8 (m).



120 **118**

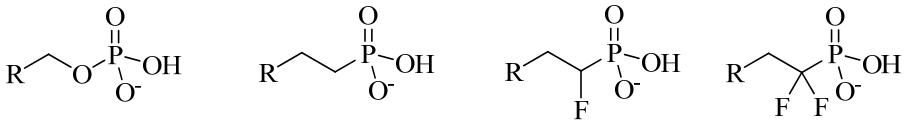
A solution of diol (32 μmol / 6 mg), NAD^+ (50 mg / 80 μmol), glycerol-1-phosphate dehydrogenase (80 U), and oxidase from *L. Sansfrancinsens* (80 U) in deuterated KP_i was gently stirred for 24 hours at room temperature. The reaction was monitored by ^{19}F NMR. After 24 hours, integration between the two diastereomers changed from 0.8 : 1 to 0.4 : 1 (Figure 3-45).



25 **26** **27** **28**

Phosphate Triester | Phosphonate | a-Fluorophosphonate | a-Difluorophosphonate

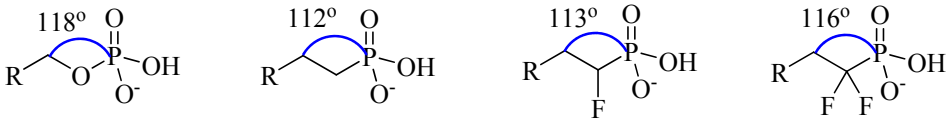
Figure 3-1. Phosphate triester, phosphonate and α-fluorinated phosphonates



29 **30** **31** **32**

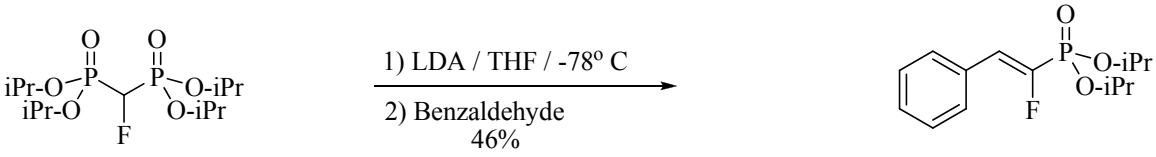
p*K*a$_2$ = 6.5 | p*K*a$_2$ = 7.5 - 8 | p*K*a$_2$ = 6.5 | p*K*a$_2$ = 5.5 - 6

Figure 3-2. Phosphate and phosphonic acid p*K*a$_2$ values



33 **34** **35** **36**

Figure 3-3. Dihedral angle comparison for phosphate and phosphonic acids



37 **38**

Figure 3-4. Bisphosphonate route to α-fluorophosphonates

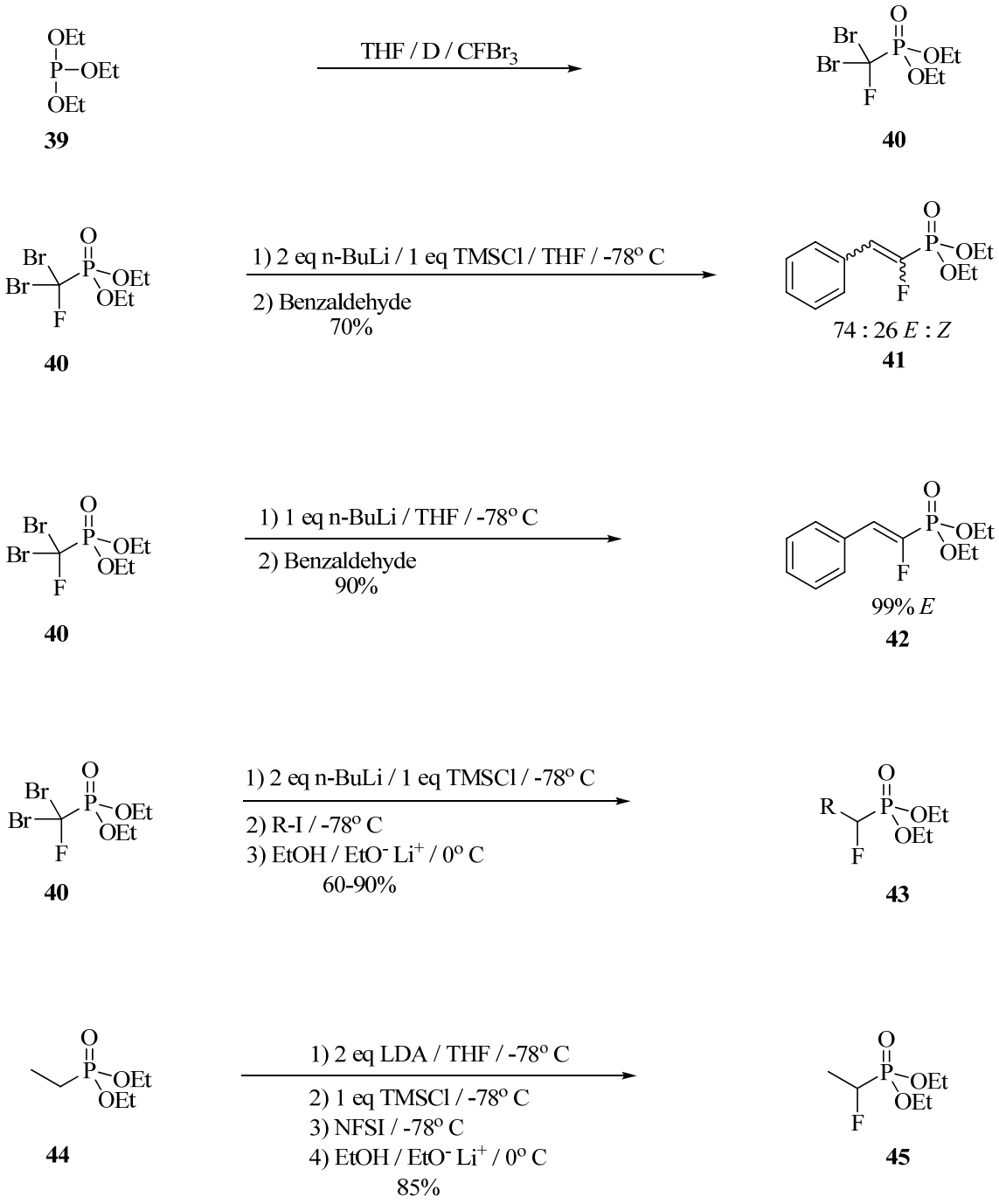


Figure 3-5. Savignac's routes to α-fluorophosphonates

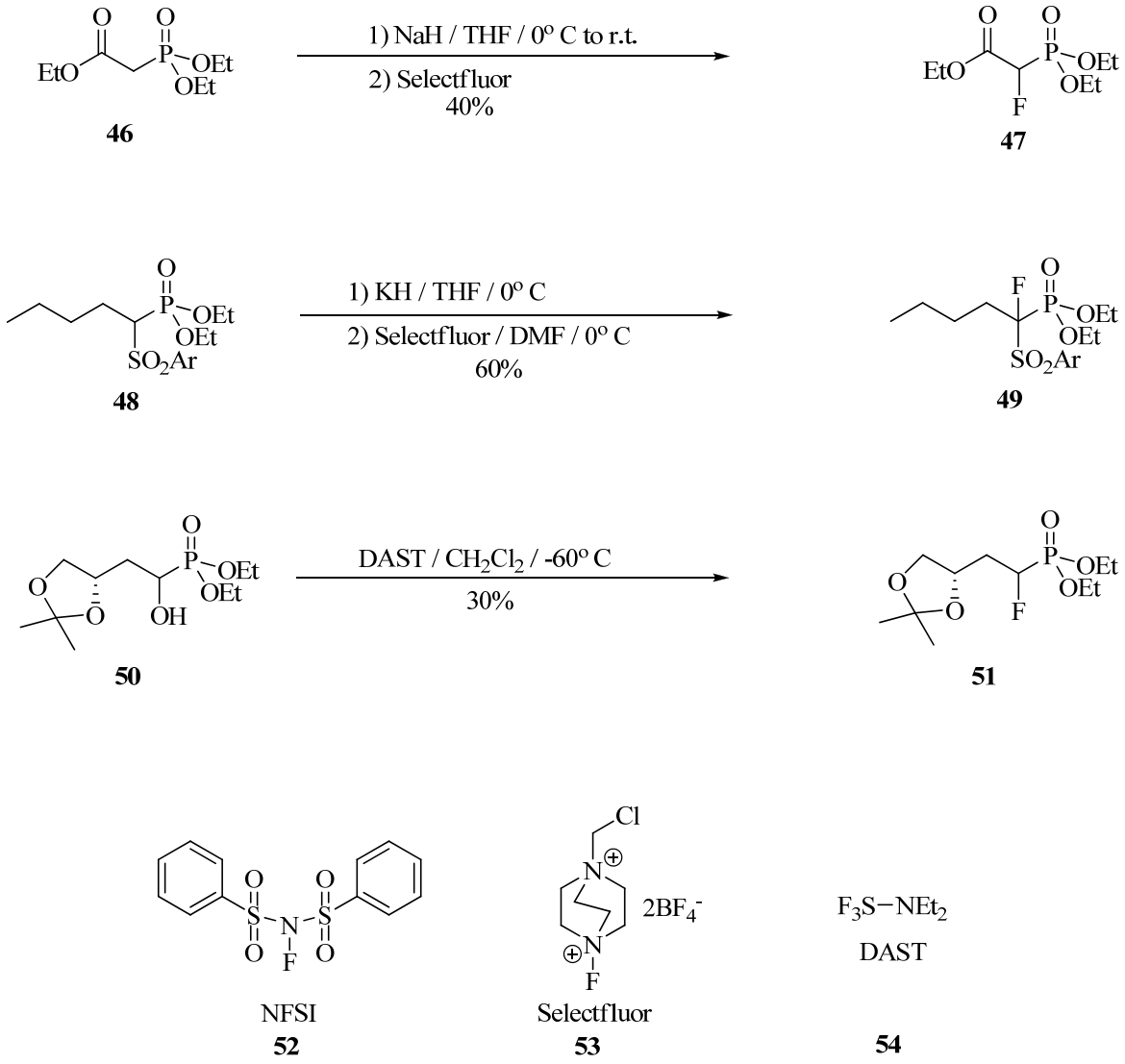


Figure 3-6. Fluorination via nucleophilic and electrophilic sources

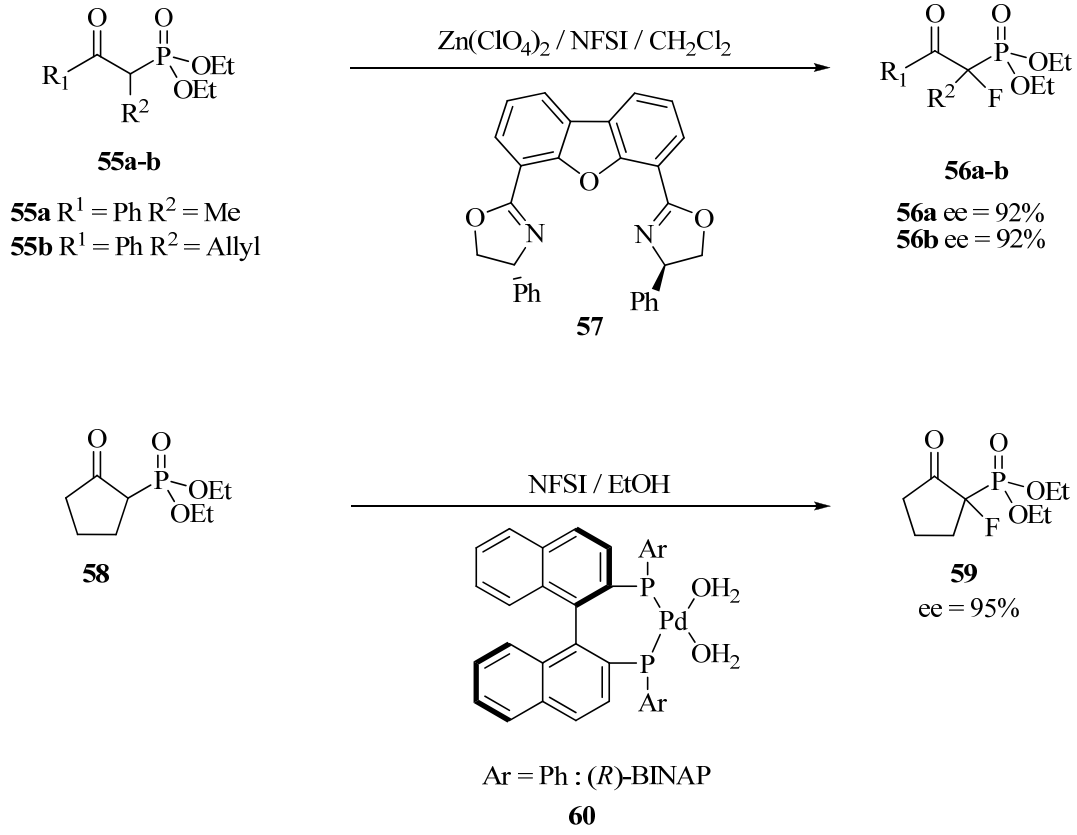
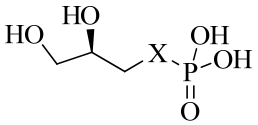
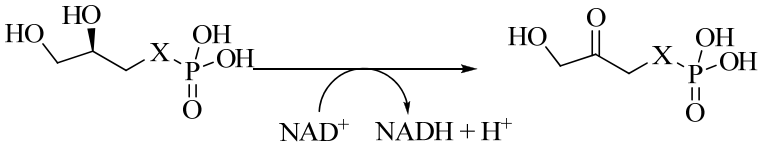


Figure 3-7. Methods for asymmetric α-fluorophosphonate synthesis



61 X = O
62 X = CH_2
63 X = CHF
64 X = CF_2

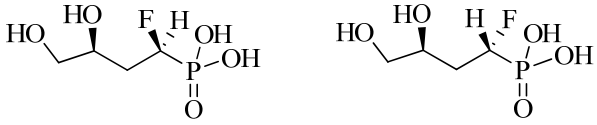
Figure 3-8. Glycerol-3-phosphate analogues synthesized by O'Hagan



61-64 **65**

Substrate	***Km***
61 X = O	0.20 mM
62 X = CH_2	0.18 mM
63 X = CHF	0.17 mM
64 X = CF_2	0.73 mM

Figure 3-9. O'Hagan's Glycerol-1-phosphate dehydrogease assay



63a **63b**

Figure 3-10. The diastereomers of **63**

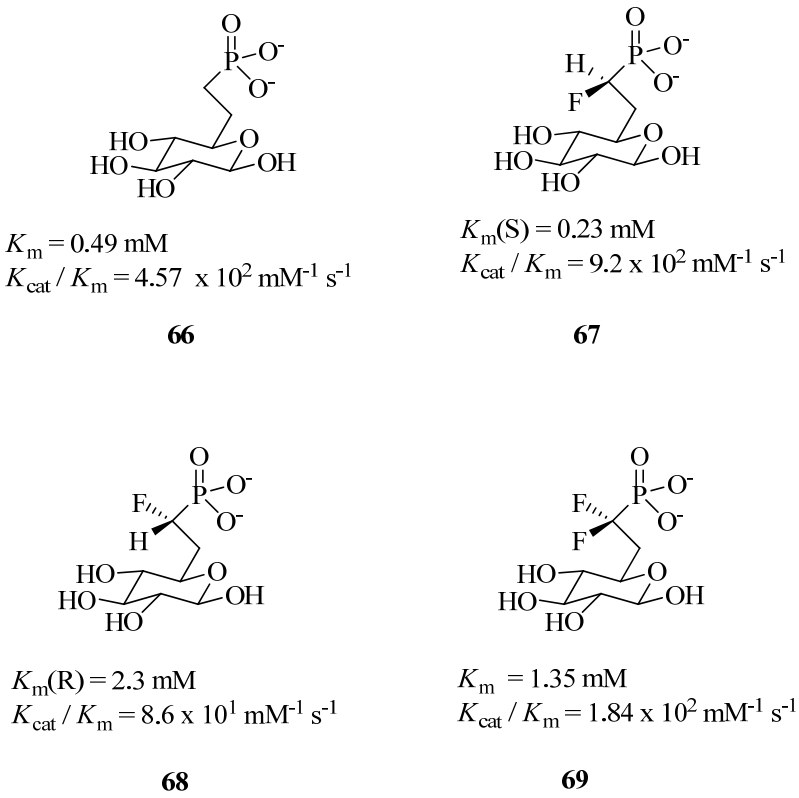


Figure 3-11. Glucose-6-phosphate analogs synthesized by Berkowitz

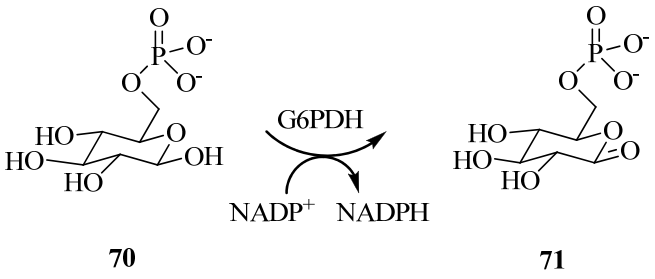
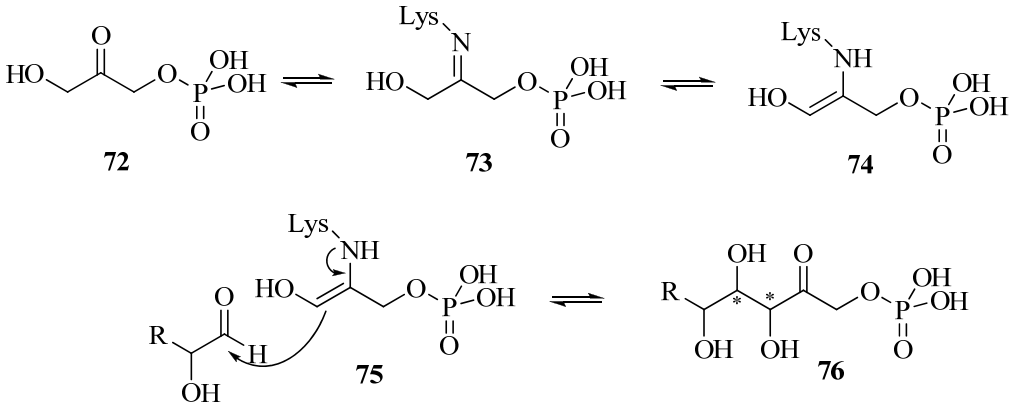


Figure 3-12. Oxidation of glucose-6-phosphate to 6-phosphogluconolactone

Class I aldolases use active site Lysine residue to form an enamine



Class II aldolases use Zn^{2+} to stabilize an enolate

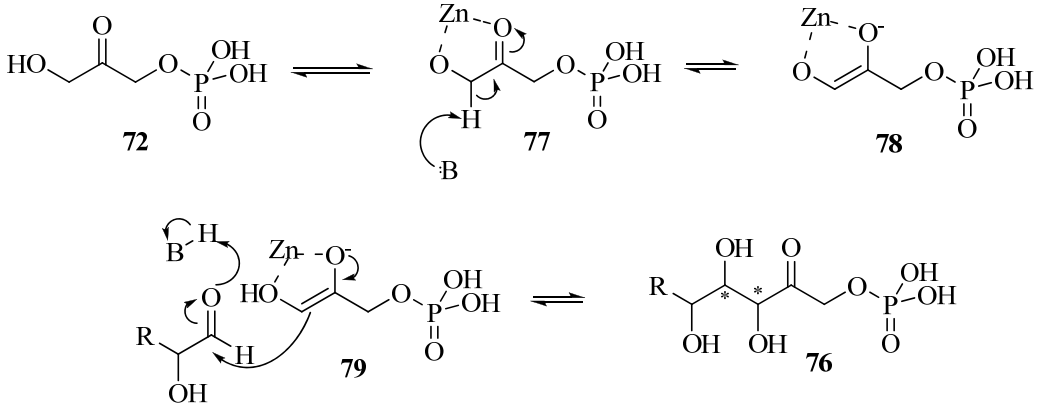


Figure 3-13. Aldol reactions with class I and class II aldolase

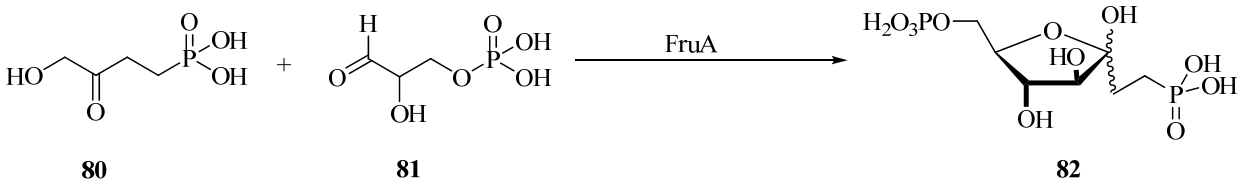


Figure 3-14. Rabbit muscle aldolase catalyzed reaction with nonnatural donor substrate **80**

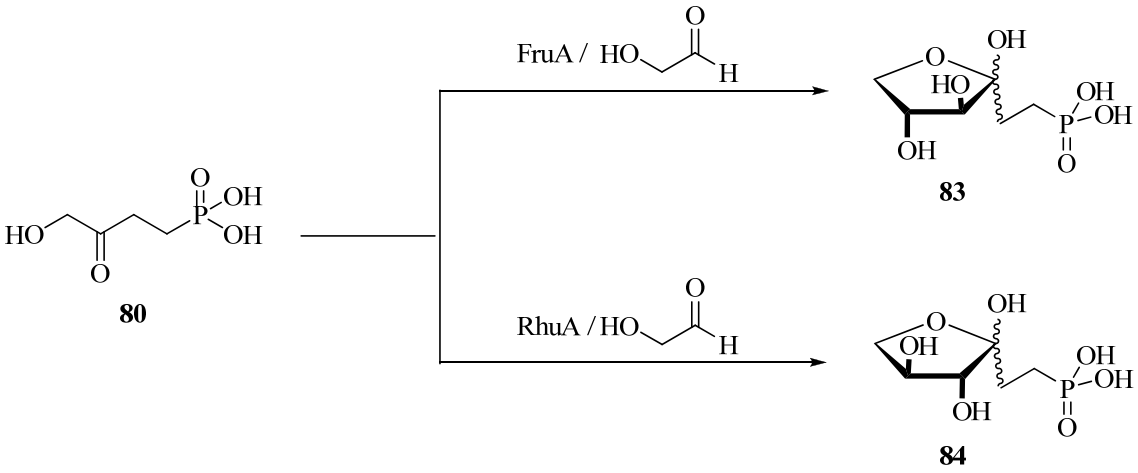


Figure 3-15. Phosphonic acid carbohydrates synthesized by Fessner

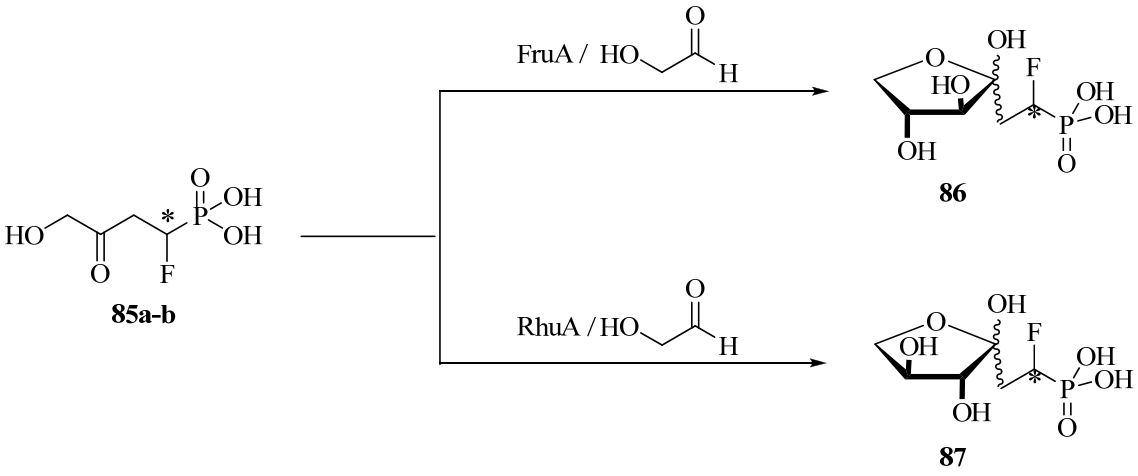


Figure 3-16. Route to α-fluorinated phosphonic acid carbohydrates

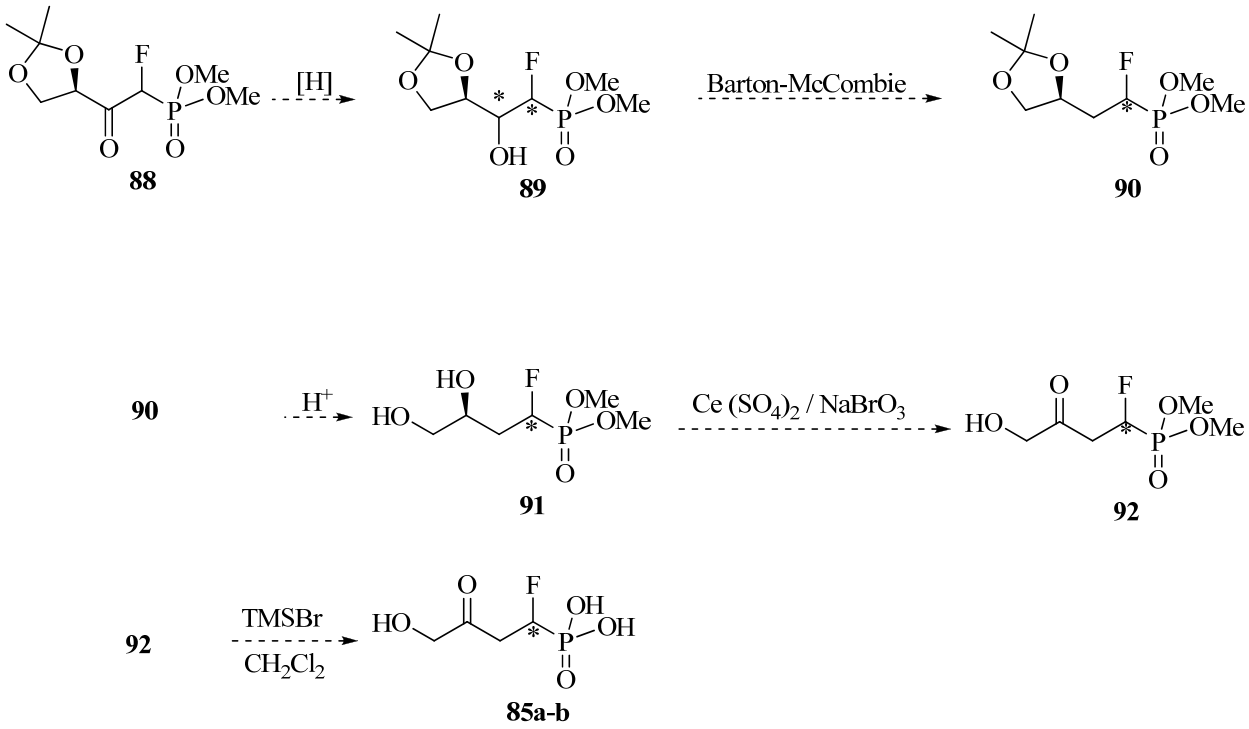


Figure 3-17. Fluorinated aldolase substrate mimics via β-ketophosphonates

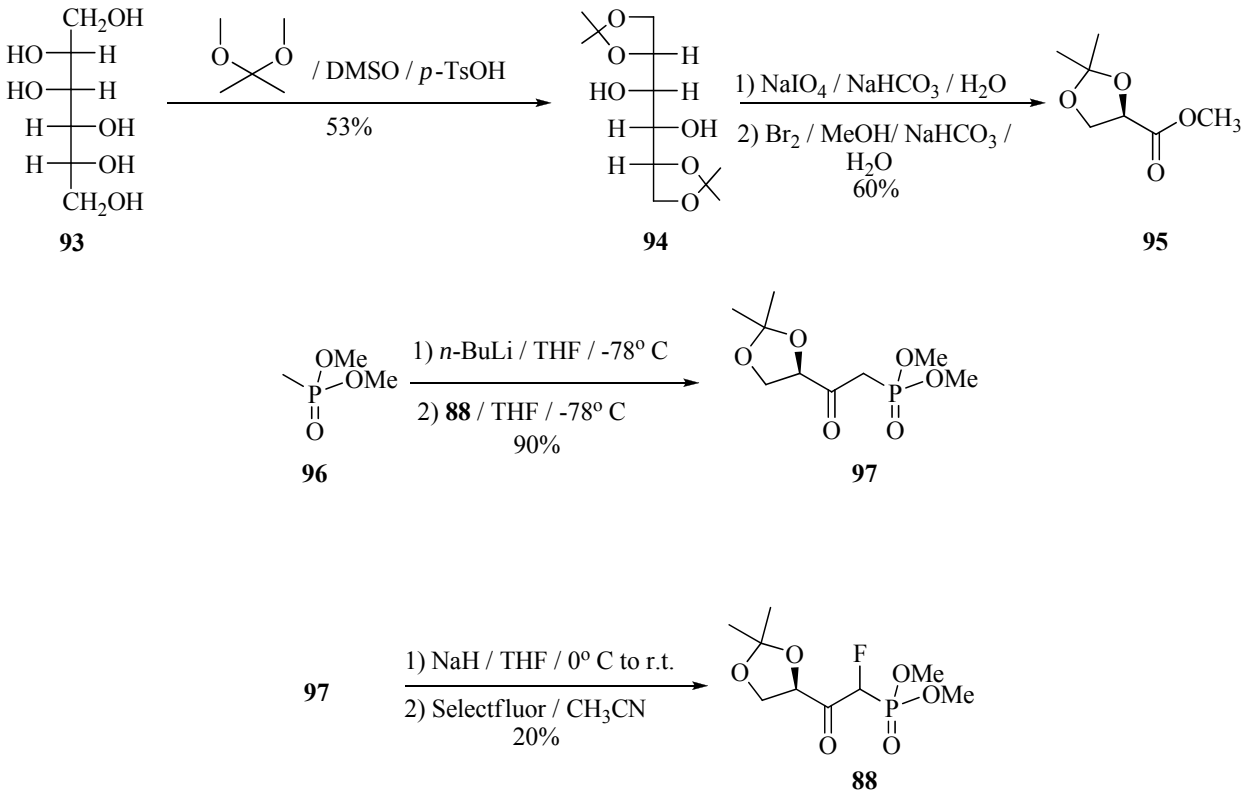


Figure 3-18. Fluorinated acetonide synthesis

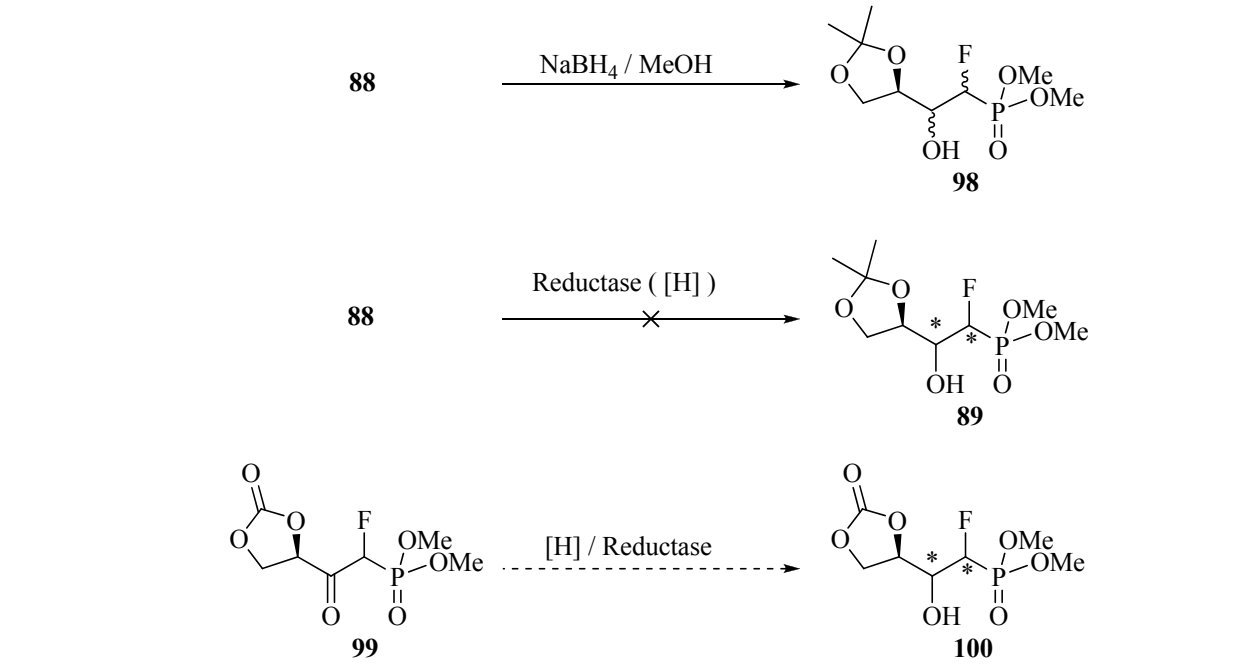


Figure 3-19. Racemic standard, reduction failure and carbonate phosphonate

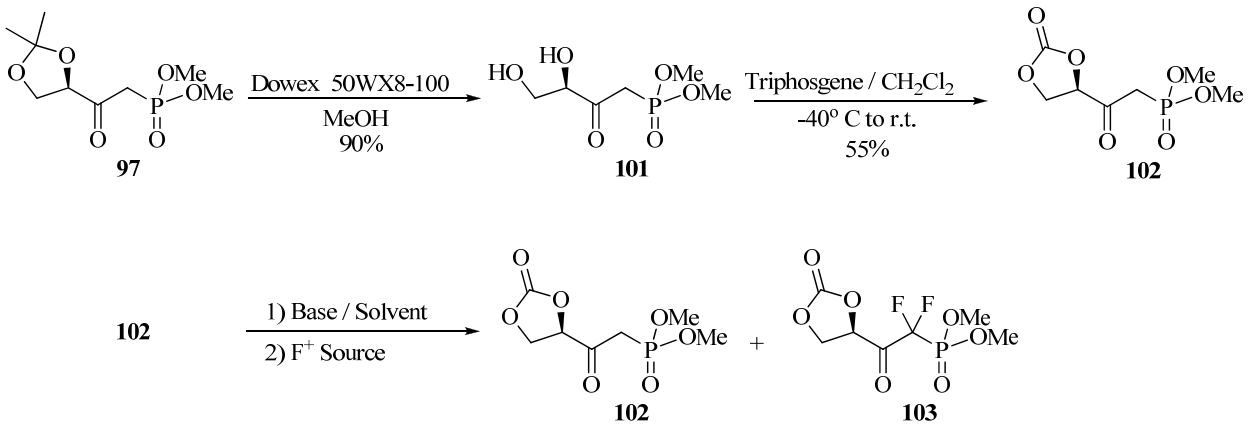


Figure 3-20. Synthesis of carbonates and subsequent difluorination

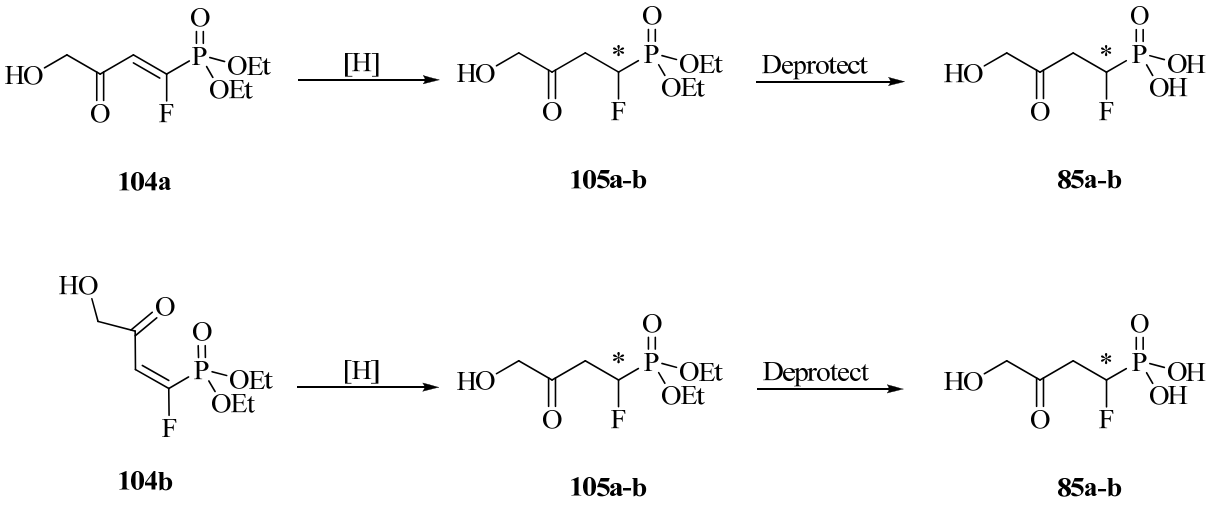


Figure 3-21. Enone reductase route to α-fluorovinylphosphonic acids

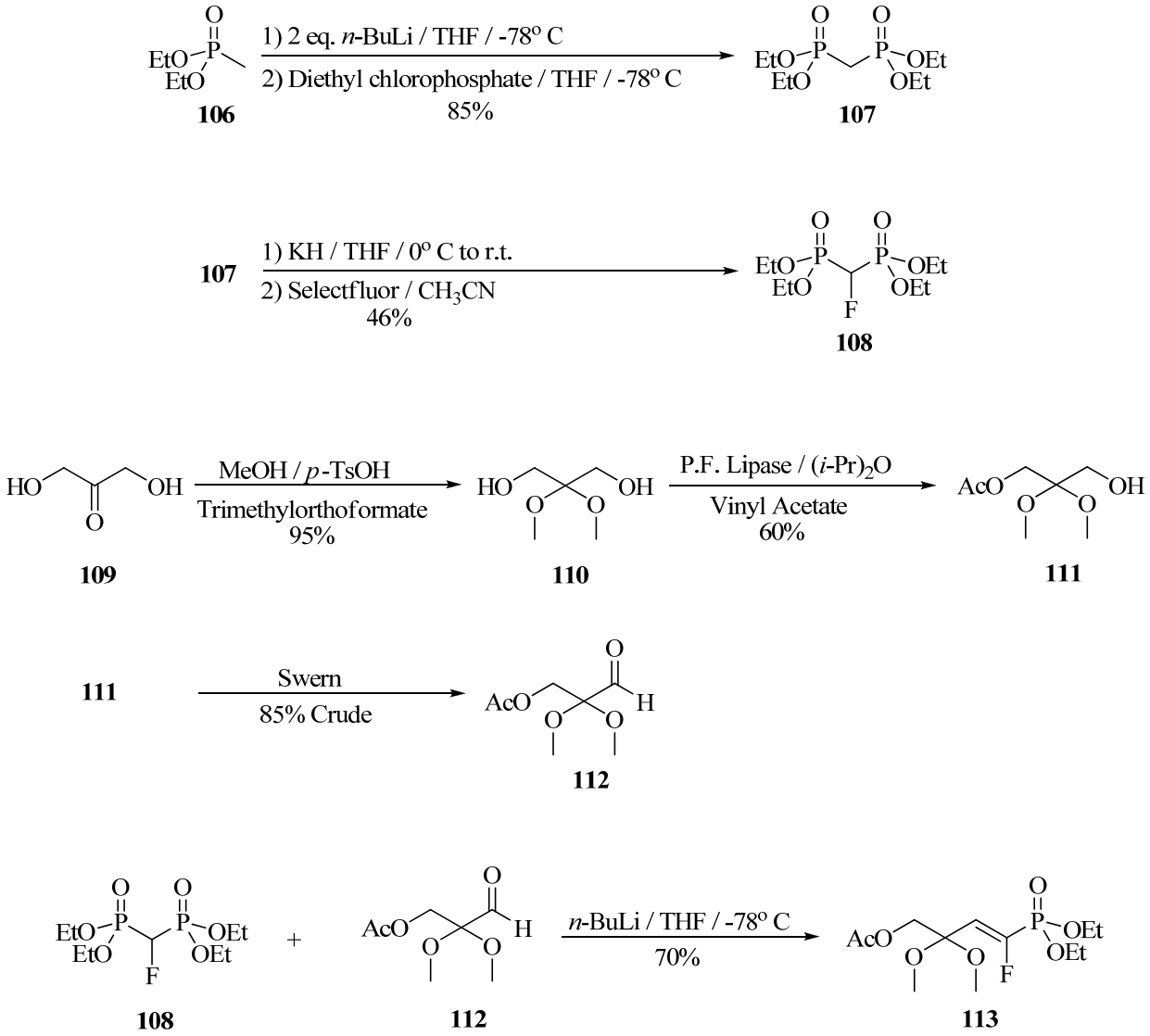


Figure 3-22. Synthesis of protected vinyl phosphonate **113**

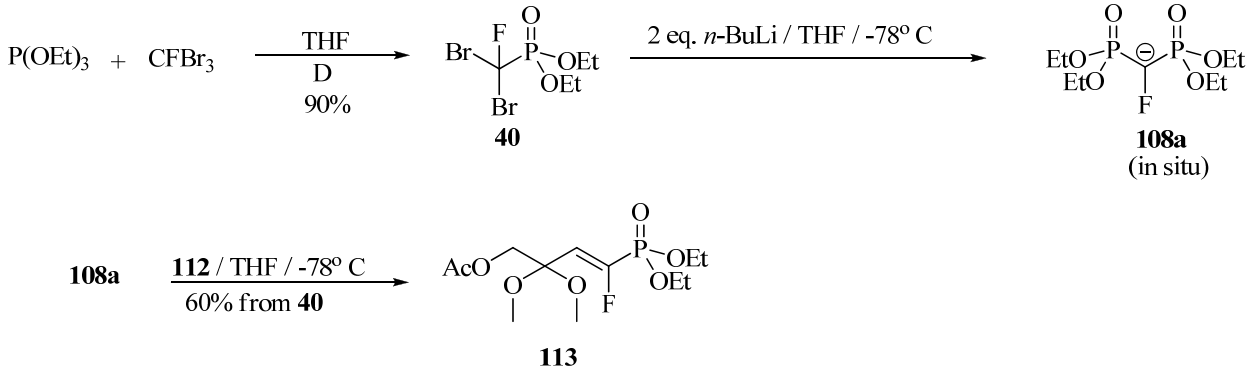


Figure 3-23. Dibromofluorophosponate route to vinyl phosphonate **113**

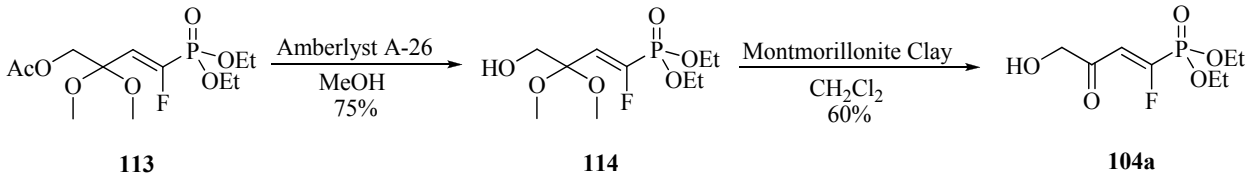


Figure 3-24. Synthesis of vinyl phosphonate **104a**

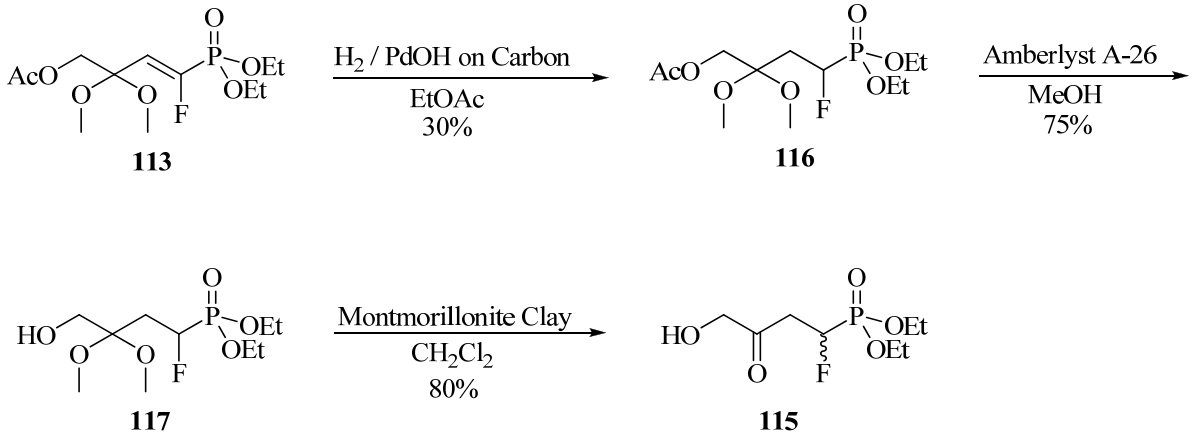


Figure 3-25. Synthesis of racemic phosphonate standard

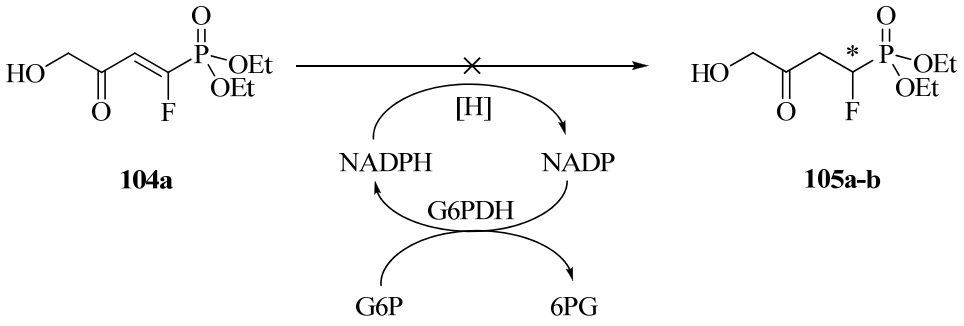


Figure 3-26. Screening of vinyl α-fluorinated phosphonate with en-reductases

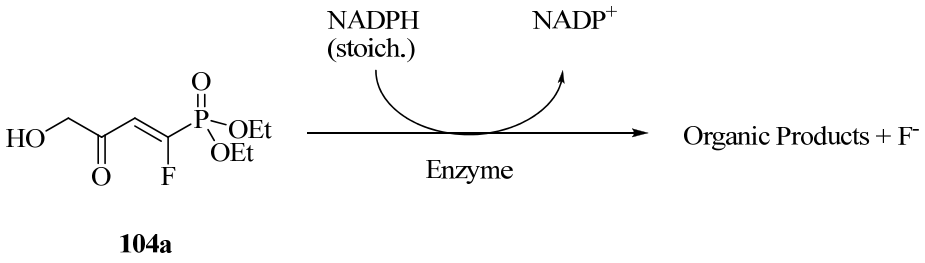


Figure 3-27. Stoichiometric reduction of vinyl phosphonate with enzyme and NADPH

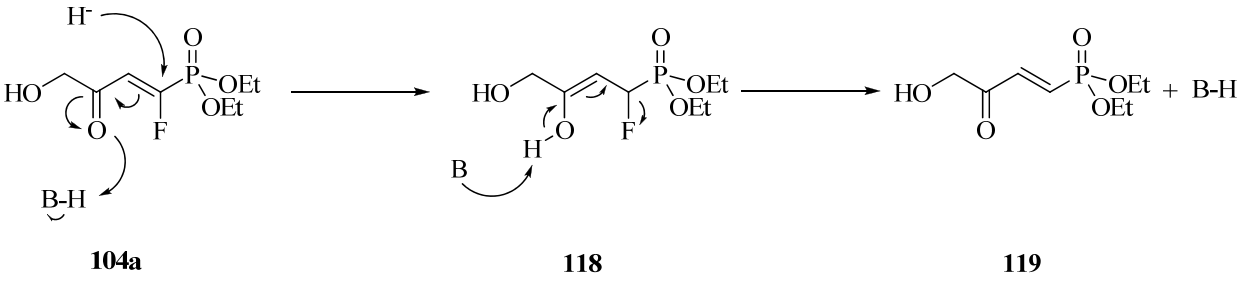


Figure 3-28. Fluorine elimination mechanism

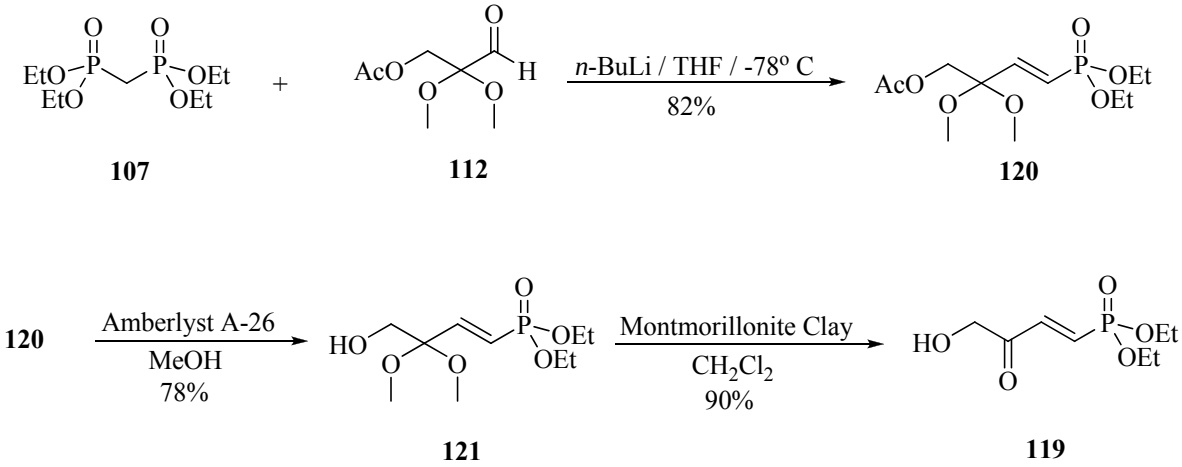


Figure 3-29. Synthesis of unfluorinated vinyl phosphonate

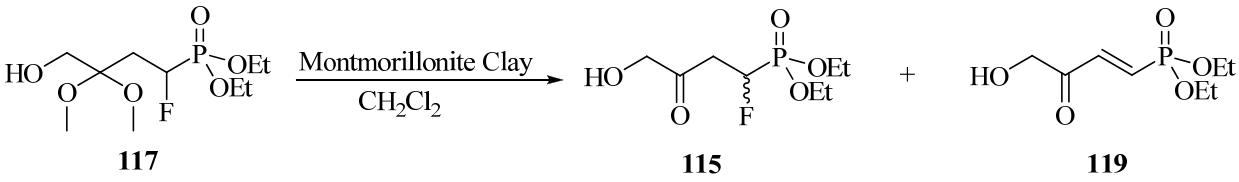


Figure 3-30. Inseparable ketal deprotection products

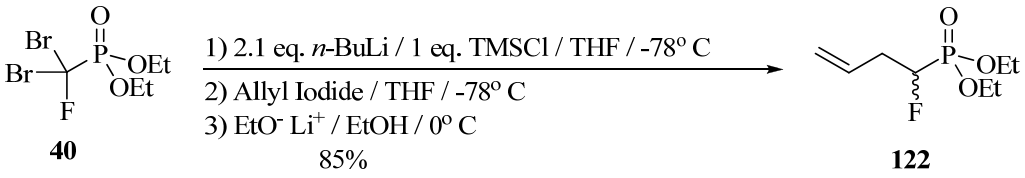


Figure 3-31. Synthesis of unsaturated phosphonate

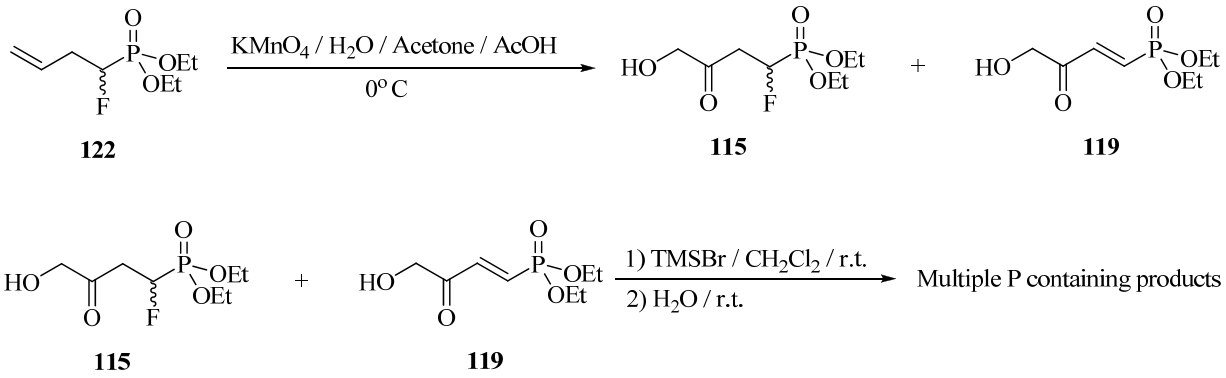


Figure 3-32. $KMnO_4$ alkene oxidation and phosphonate deprotection

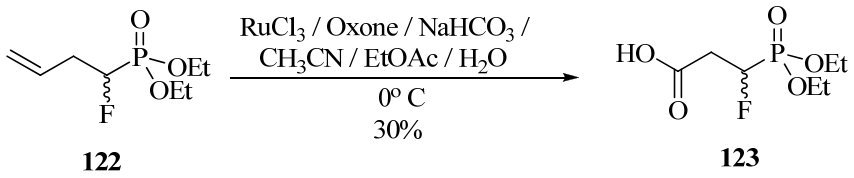


Figure 3-33. RuO_4 catalyzed alkene oxidation and phosphonate deprotection

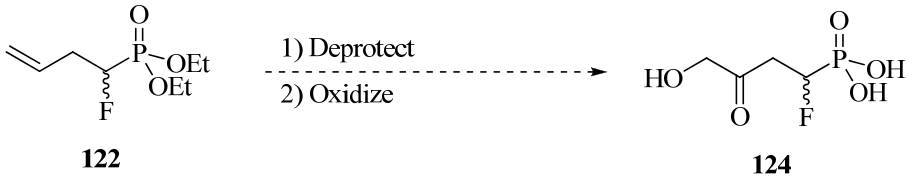


Figure 3-34. Route to α-hydroxyketone via initial phosphonate deprotection

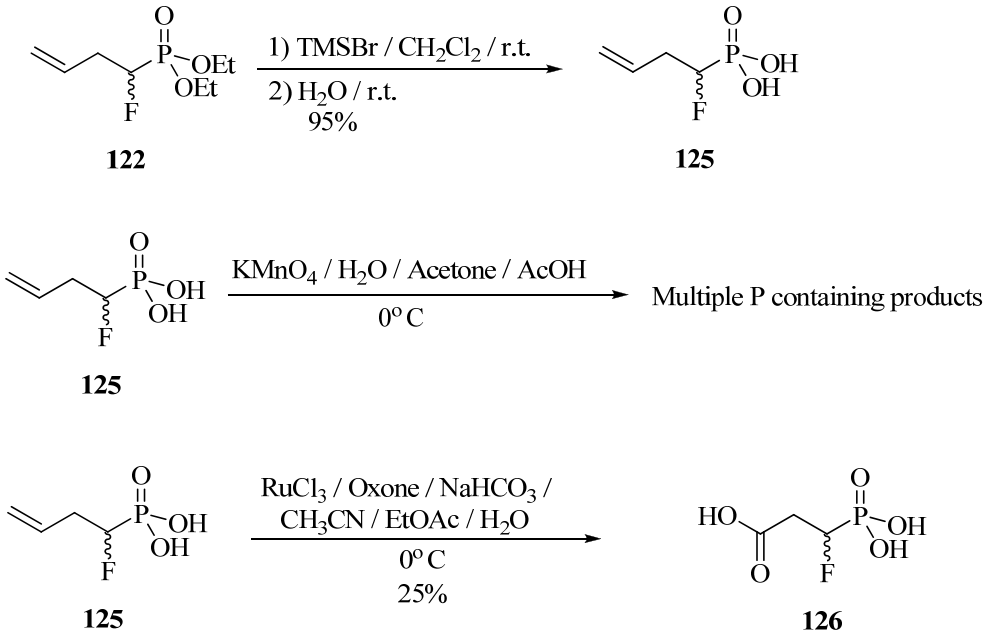


Figure 3-35. Alkene oxidation of phosphonic acid **125**

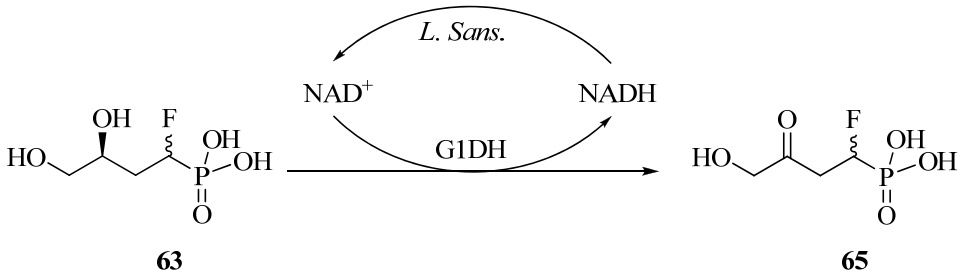


Figure 3-36. Selective diol oxidation of phosphonic acid **63**

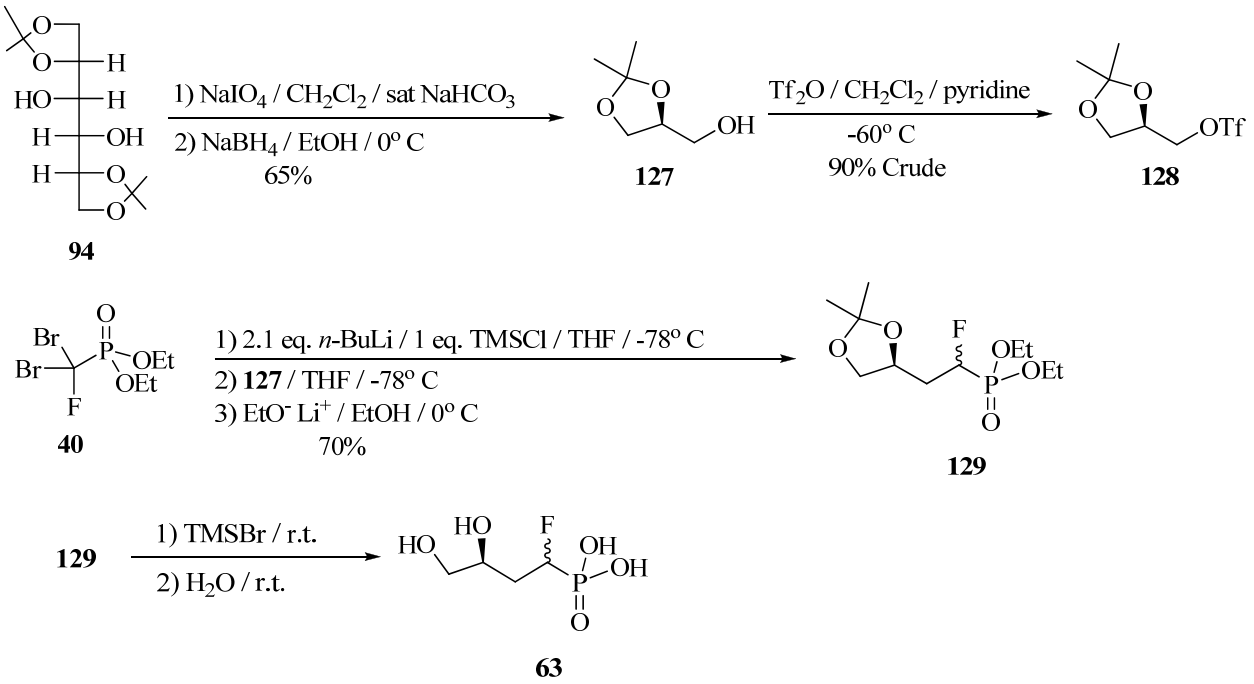


Figure 3-37. Synthetic route to diol **63**

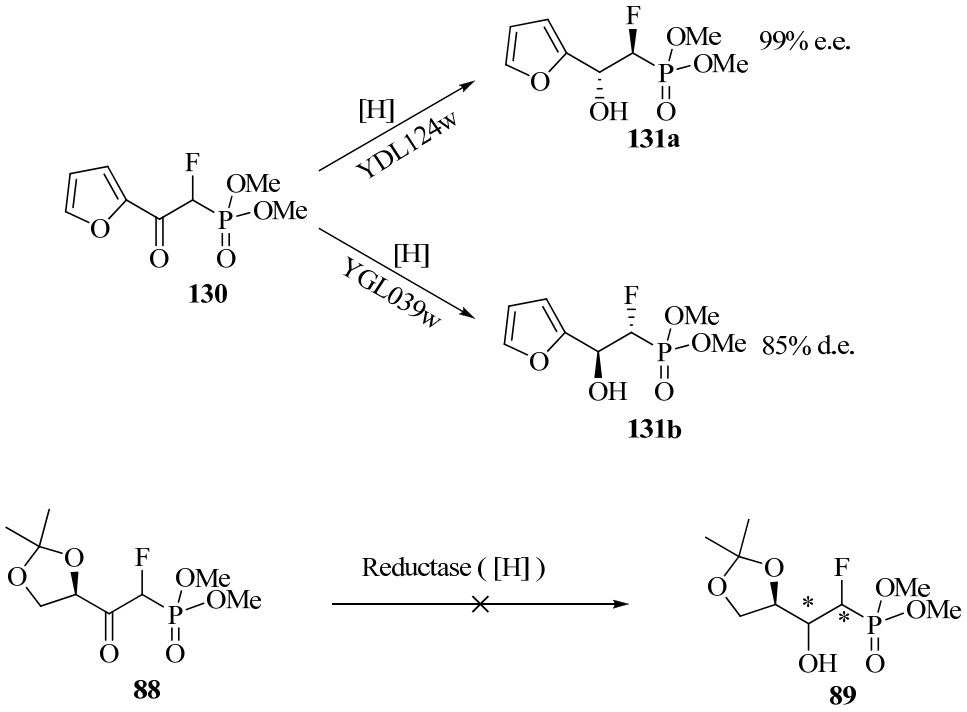


Figure 3-38. Reduction of α-fluoro-β-ketophosphonate **130** with purified enzymes

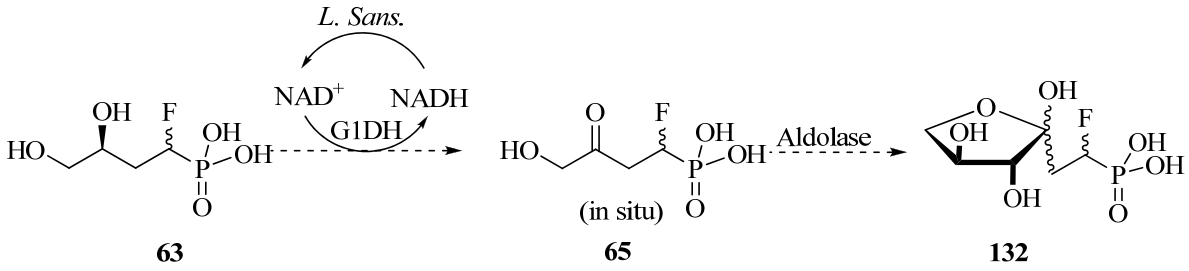
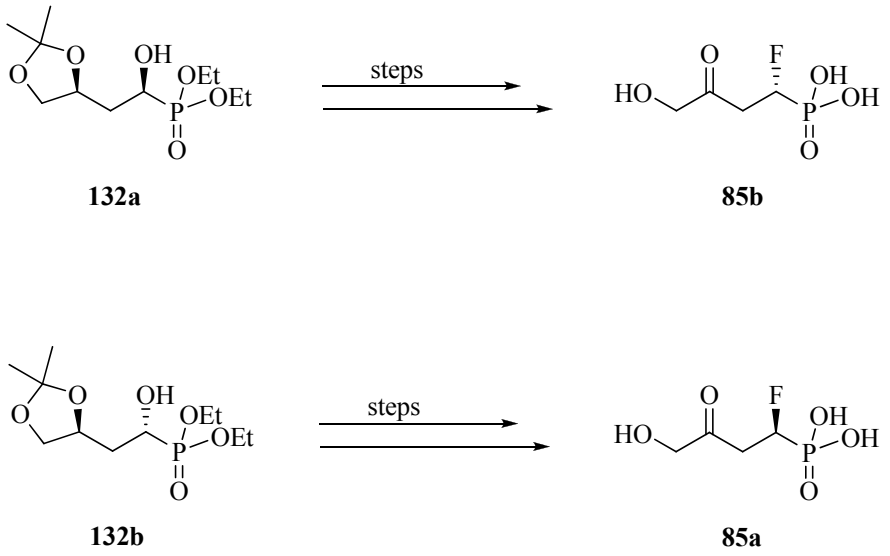


Figure 3-39. Oxidation of diol **63** followed by in situ aldolase reaction



132a **85b**

132b **85a**

Figure 3-40. Future route to optically pure α-fluorophosphonic acids

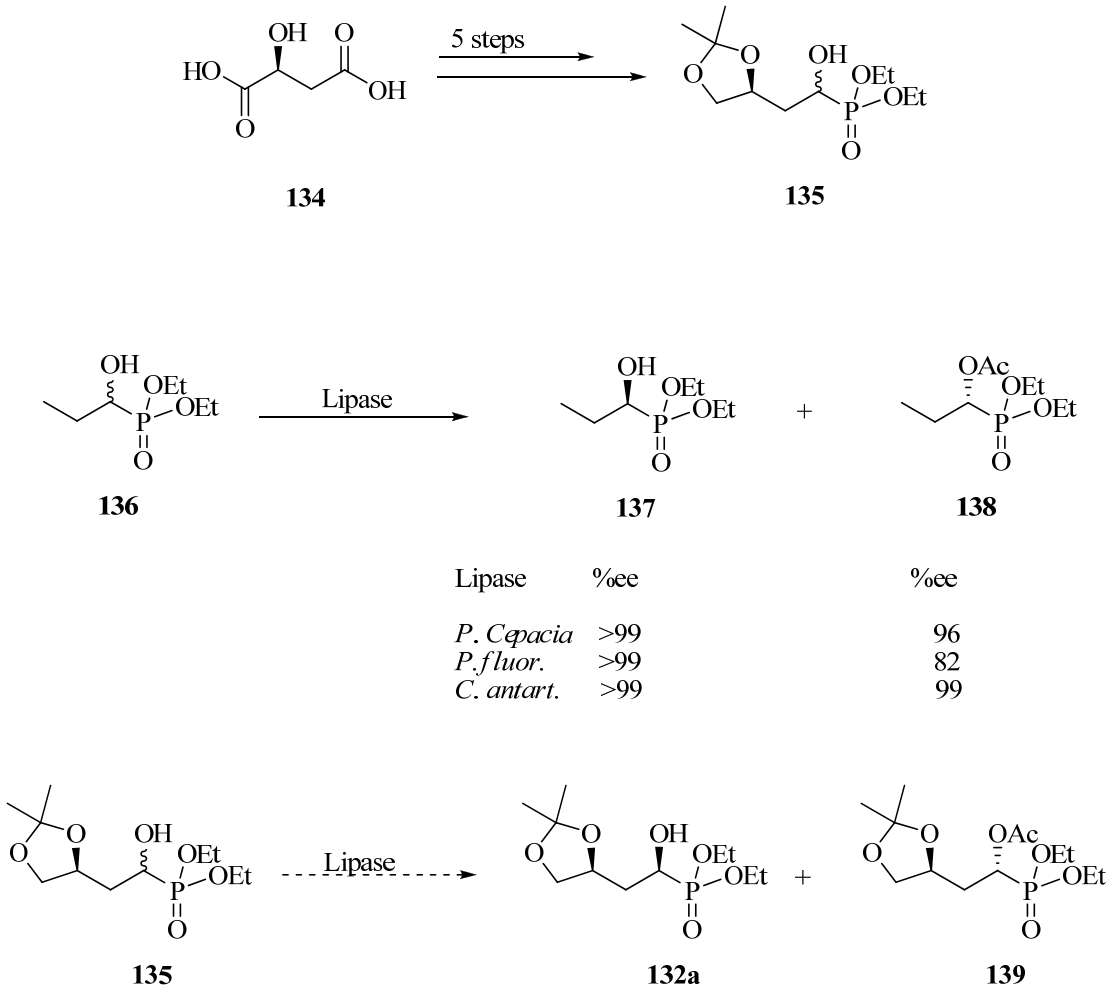


Figure 3-41. Future synthetic methods

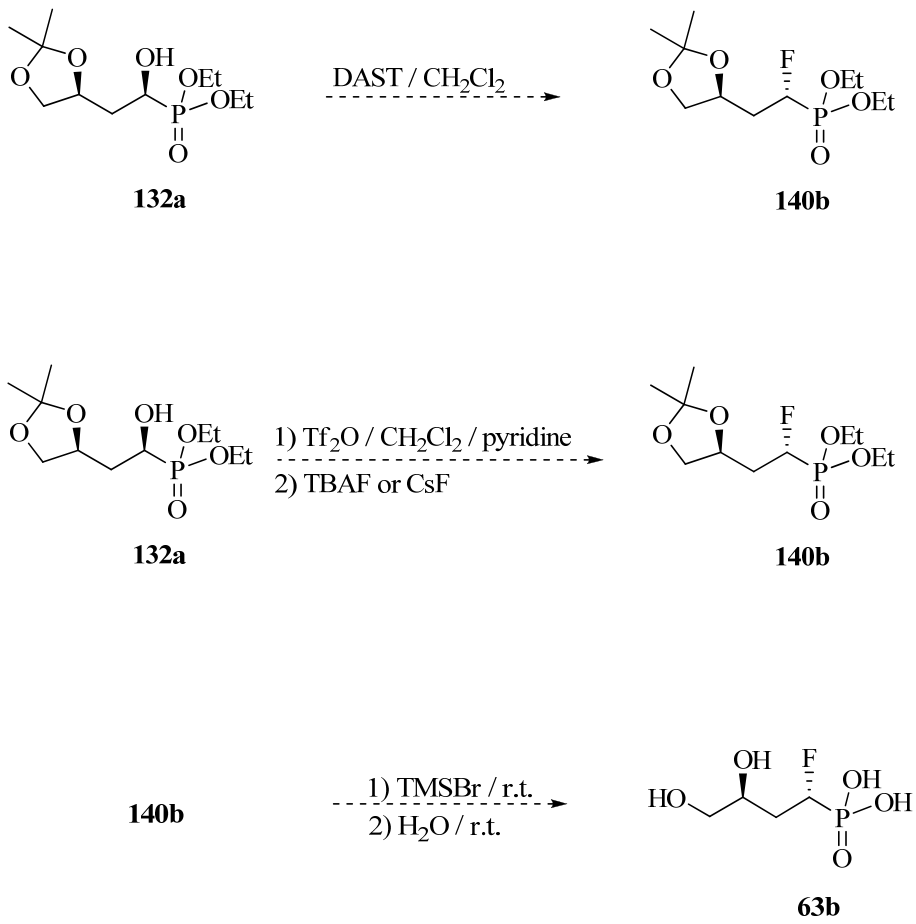


Figure 3-42. Proposed synthesis of optically pure α-fluorophosphonate **63b**

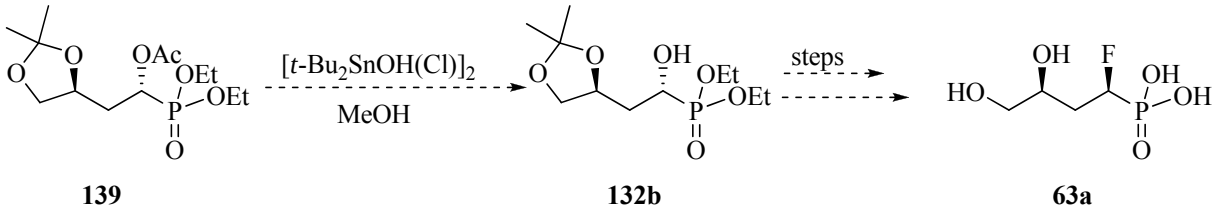


Figure 3-43. Proposed synthesis of α-fluorophosphonic acid **63a**

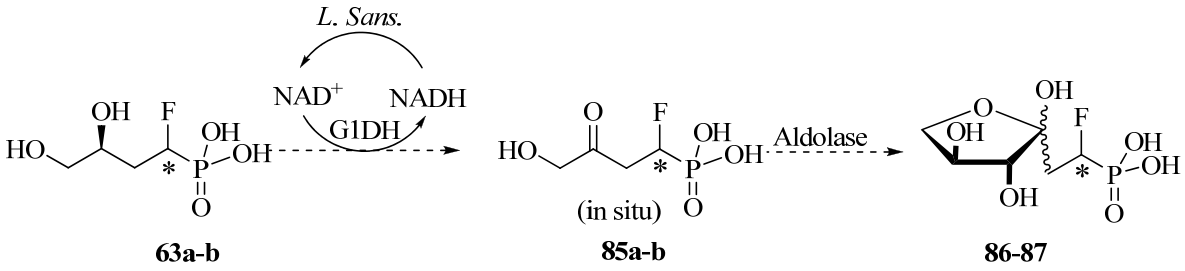


Figure 3-44. Proposed synthesis of α-fluorophosphonic acid carbohydrates

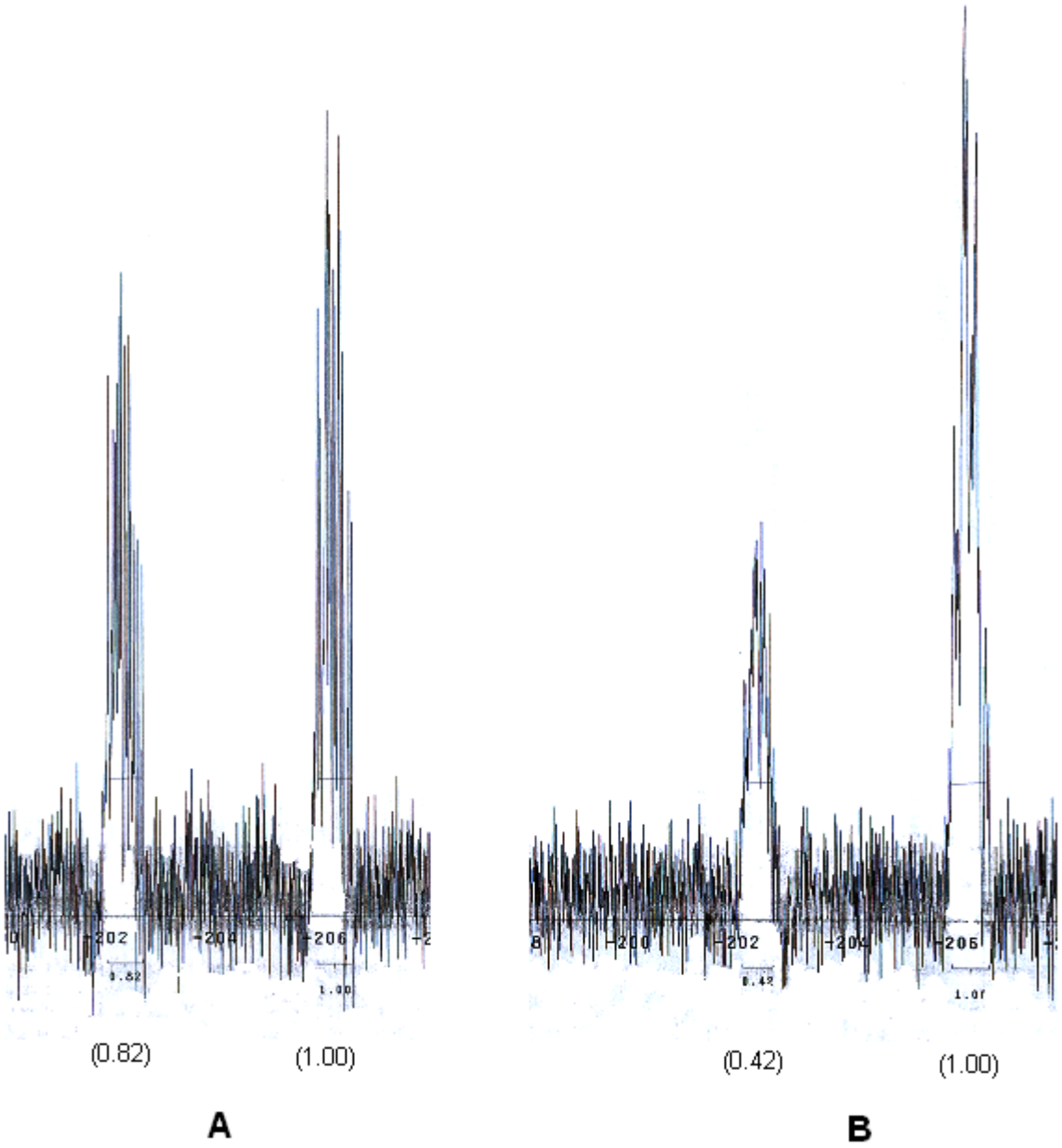


Figure 3-45. Decrease in starting material over a 24 hour period

Table 3-1. Fluorination conditions of carbonate **102**

Base / Solvent / Temperature	**F^+ Agent / Solvent / Temperature**
NaH / THF / 0° C	Selectfluor / THF / 0° C
NaH / THF / 0° C	Selectfluor / CH_3CN / 0° C
NaH / CH_3CN / 0° C	Selectfluor / CH_3CN / 0° C
NaH / CH_3CN / -10° C	2 equiv. Selectfluor / CH_3CN / -10° C
LDA / THF / -78° C to -10° C	Selectfluor / THF / -10° C
LDA / THF / -78° C to -10° C	Selectfluor / CH_3CN / -10° C
KH / THF / -10° C	Selectfluor / CH_3CN / -10° C
NaH / THF / -10° C	NFSI / THF / -10° C
LDA / THF / -78°C to -10° C	NFSI / THF / -10° C
KH / THF / -10° C	NFSI / THF / -10° C

REFERENCE LIST

(1) Frater, G. *Helv. Chim. Acta* **1979**, *62*, 2825-2828

(2) Shieh, W. R.; Gopalin, A. S.; Sih, C. J. *J. Am. Chem. Soc.* **1985**, *107*, 2993-2994.

(3) Shieh, W. R.; Sih, C. J. *Tetrahedron: Asymmetry* **1993**, *4*, 1259-1269.

(4) Nakamura, K.; Kawai, Y.; Nakajima, N.; Harada, T. *J. Org. Chem.* **1991**, *56*, 4778-4783.

(5) Kaluzna, I. A.; Matsuda, T.; Sewell, A. K.; Stewart, J. D. *J. Am. Chem. Soc.* **2004**, *126*, 12827-12832.

(6) Feske, B. D.; Kaluzna, I. K.; Stewart, J. D. *J. Org. Chem.* **2005**, *70*, 9654-9657.

(7) Feske, B. D.; Stewart, J. D. *Tetrahedron: Asymmetry* **2005**, *16*, 3124-3127.

(8) Kaluzna, I. A.; Feske, B. D.; Wittayanan, W.; Ghiviriga, I.; Stewart, J. D. *J. Org. Chem.* **2005**, *70*, 342-345.

(9) Buisson, D.; Sanner, C.; Larcheveque, M.; Azerad, R. *Tetrahedron Lett.* **1987**, *28*, 3939-3940.

(10) Larcheveque, M.; Henrot, S. *Tetrahedron* **1990**, *46*, 4277-4282.

(11) Hena, M. A.; Terauchi, S.; Kim, C.; Horiike, M.; Kiyooka, S. *Tetrahedron: Asymmetry* **1998**, *9*, 1883-1890.

(12) Smith, A. B.; Levenberg, P. A. *Synthesis* **1981**, *7*, 567-570.

(13) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647-2649.

(14) Kiyooka, S. *Tetrahedron: Asymmetry* **2003**, *14*, 2897-2910.

(15) Bellec, C.; Gaurat, O. *Can. J. Chem.* **2004**, *82*, 1289-1293.

(16) Katrizky, A. R.; Wang, Z.; Wang, M.; Wilkerson, C.; Hall, D.; Novruz, G. A. *J. Org. Chem.* **2004**, *69*, 6617-6622.

(17) Nagao, K.; Chiba, M.; Kim, S. W. *Synthesis* **1983**, 197-199.

(18) Puntambekar, H. M.; Naik, D. G. *Synth. Comm.* **1998**, *28*, 2399-2406.

(19) Kalaitzakis, D.; Kambourakis, S.; Rozzell, D.; Smonou, I. *Tetrahedron: Asymmetry* **2007**, *18*, 2418-2426.

(20) Seco, J. M.; Quinoa, E.; Riguera, R. *Tetrahedron: Asymmetry* **2001**, *12*, 2915-2925.

(21) Seco, J. M.; Quinoa, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17-117.

(22) Oppolzer, W.; Marco-Contelles, J. *Helv. Chim. Acta* **1986**, *69*, 1699-1703.

(23) Kalaitzakis, D.; Rozzell, J. D.; Kambourakis, S.; Smonou, I. *Org. Lett.* **2005**, *7*, 4799-4801.

(24) Kim, J.; Oh, W.; Kang, S. *J. Korean Chem. Soc.* **1991**, *35*, 589-591.

(25) Irwin, W. *New York Tribune* **1915**.

(26) Olson, K. *Emerging Infect. Diseases* **1999**, 513-516.

(27) Minami, M.; Hui, D.; Katsumata, M.; Inagaki, H.; Boulet, C. *J. Chrom. B* **1997**, *695*, 237-244.

(28) Shih, M. L.; Smith, J. R.; McMonagle, J. D.; Dolzine, T. W.; Gresham, V. C. *Biol. Mass Spectrom.* **1991**, *20*, 717-725.

(29) Noort, D.; Hulst, A. G.; Platenburg, D. H. J.; M., P.; Benschop, H. *Archiv. Toxicol.* **1998**, *72*, 671-675.

(30) Black, R. M.; Read, R. W. *J. Chrom. A* **1998**, *794*, 233-244.

(31) Klug, S. J.; Famulok, M. *Mol. Biol. Rep.* **1994**, *20*, 97-107.

(32) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505-510.

(33) Joyce, G. F. *Gene* **1989**, *82*, 83-87.

(34) Famulok, M.; Szostak, J. W. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 979-988.

(35) Shangguan, D.; Ying, L.; Zhiwen, T.; Cao, Z. C.; Chen, H. W.; Mallikaratchy, P.; Sefah, K.; Yang, C. J.; Tan, W. *Pub. Nat. Acad. Sci.* **2006**, *103*, 11838-11843.

(36) Mann, D.; Reinemann, C.; Stoltenburg, R.; Strehlitz, B. *Biochem. Biophys. Res. Comm.* **2005**, *338*, 1928-1934.

(37) Filho, E. P.; Rodrigues, A. R.; Moran, P. J. *Tetrahedron: Asymmetry* **2001**, *12*, 847-852.

(38) Nakajima, N.; Sasaki, K.; Ozawa, H.; Sekijima, Y.; Morita, H.; Fukushima, Y.; Yanagisawa, N. *Archiv. Toxicol.* **1998**, *72*, 601-603.

(39) Stoltenburg, R.; Reinemann, C.; Strehlitz, B. *Anal. Bioanal. Chem.* **2005**, *383*, 83-91.

(40) McKay, A. F.; Braun, R. O.; Vavasour, G. R. *J. Am. Chem. Soc.* **1952**, *74*, 5540-5541.

(41) De Luca, L.; Giacomelli, G.; Masala, S.; Porcheddu, A. *J. Org. Chem.* **2003**, *68*, 4999-5001.

(42) Desai, M. C.; Stramiello, L. M. *Tetrahedron Lett.* **1993**, *34*, 7685-7688.

(43) Ladame, S.; Willson, M.; Perie, J. *Eur. J. Org. Chem.* **2002**, 2640-2648.

(44) Blackburn, G. M. *Chem. Ind.* **1981**, 134-138.

(45) Ruiz, M.; Ojea, V.; Quintela, J. M.; Guillin, J. J. *Chem. Comm.* **2002**, 1600-1601.

(46) Nieschalk, J.; Batsanov, A. S.; O'Hagan, D.; Howard, J. A. *Tetrahedron* **1996**, *52*, 165-176.

(47) Dunitz, J. D.; Taylor, R. *Chem. Eur. J.* **1997**, *3*, 89-98.

(48) Howard, J. A.; Hoy, V. J.; O'Hagan, D.; Smith, G. T. *Tetrahedron* **1996**, *52*, 12613-12622.

(49) Blackburn, G. M.; Parratt, M. J. *J. Chem. Soc. Chem. Comm.* **1982**, 1270-1271.

(50) Xu, Y.; Qian, L.; Prestwich, G. D. *Org. Lett.* **2003**, *5*, 2267-2270.

(51) Iorga, B.; Eymery, F.; Savignac, P. *Tetrahedron Lett.* **1998**, *39*, 4477-4480.

(52) Waschbusch, R.; Carran, J.; Savignac, P. *Tetrahedron* **1996**, *52*, 14199-14216.

(53) Waschbusch, R.; Carran, J.; Savignac, P. *J. Chem Soc. Perk. Trans. 1* **1997**, 1135-1139.

(54) Burton, D. I.; Flynn, R. M. *J. Fluorine Chem.* **1977**, *10*, 329-334.

(55) Lal, G. S. *J. Org. Chem.* **1993**, *58*, 2791-2796.

(56) Marma, M. S.; Khawli, L. A.; Harutunian, V.; Kashemirov, B. A.; McKenna, C. E. *J. Fluorine Chem.* **2005**, *126*, 1467-1475.

(57) Wnuk, S. F.; Robins, M. J. *J. Am. Chem. Soc.* **1995**, *60*, 7902-7908.

(58) Xu, Y.; Qian, L.; Prestwich, G. *J. Org. Chem.* **2003**, *68*, 5320-5330.

(59) Singh, R. P.; Shreeve, J. M. *Synthesis* **2002**, 2561-2578.

(60) Bernardi, L.; Jorgensen, K. A. *Chem. Comm.* **2005**, *10*, 1324-1326.

(61) Hamashima, Y.; Suzuki, T.; Takano, H.; Shimura, Y.; Tsuchiya, Y.; Moriya, K.; Goto, T.; Sodeoka, M. *Tetrahedron* **2006**, *62*, 7168-7179.

(62) Berkowitz, D. B.; Bose, M.; Pfannenstiel, T. J.; Doukov, T. *J. Org. Chem.* **2000**, *65*, 4498-4508.

(63) Stribling, D. *Biochem. J.* **1974**, *141*, 725-728.

(64) Arth, H. L.; Fessner, W. D. *Carb. Res.* **1998**, *305*, 313-321.

(65) Ladame, S.; Bardet, M.; Perie, J.; Willson, M. *Bioorg. Med. Chem.* **2001**, *9*, 773-783.

(66) Doyle, M. P.; Hu, W.; Phillips, I. M.; Moody, C. J.; Pepper, A. G.; Slawin, A. G. *Adv. Synth. Catal.* **2001**, *343*, 112-117.

(67) Park, K. H.; Yoon, Y. J.; Lee, S. G. *Tetrahedron Lett.* **1994**, *35*, 9737-9739.

(68) Majer, P.; Ramnayayan, S. R. *J. Org. Chem.* **1994**, *59*, 1937-1938.

(69) Bougioukou, D. J.; Stewart, J. D. *J. Am. Chem. Soc.* **2008**, *130*, 7655-7658.

(70) Teulade, M. P.; Savignac, P.; Aboujaoude, E. E.; Lietge, S.; Collignon, N. *J. Organometallic Chem.* **1986**, *304*, 283-300.

(71) Zupan, M. *J. Fluorine Chem.* **1976**, *8*, 305-310.

(72) Charmantray, F.; Lahssen, E. B.; Gefflaut, T.; Hecquet, L.; Bolte, J.; Lemaire, M. *J. Org. Chem.* **2004**, *69*, 9310-9312.

(73) Page, P.; Blonski, C.; Perie, J. *Tetrahedron Lett.* **1996**, *36*, 8027-8033.

(74) Gautier, E. C.; Graham, A. E.; McKillop, A.; Standen, S. P.; Taylor, R. K. *Tetrahedron Lett.* **1997**, *38*, 1881-1884.

(75) Suzuki, H.; Aly, S. M.; Wataya, Y.; Kim, H. S.; Tamai, I.; Kita, M.; Uemura, D. *Chem. Pharm. Bull.* **2007**, *55*, 821-825.

(76) Konosu, T.; Oida, S. *Chem. Pharm. Bull.* **1999**, *39*, 2212-2215.

(77) Cassel, S.; Debaig, C.; Benvegnu, T.; Chaimbault, P.; Lafosse, M.; Plusqeullec, D.; Rollin, P. *Eur. J. Org. Chem.* **2001**, 875-896.

(78) Pamies, O.; Backvall, J. E. *J. Org. Chem.* **2003**, *68*, 4815-4818.

(79) Tewson, T. J.; Welch, M. J. *J. Org. Chem.* **1978**, *43*, 1090-1092.

(80) Chorgade, M. S.; Mohapatra, D. K.; Sahoo, G.; Gurjar, M. K.; Mandlecha, M. V.; Bhoite, N.; Moghe, S.; Raines, R. T. *J. Fluorine Chem.* **2008**, *129*, 781-784.

(81) Orita, A.; Hamada, Y.; Nakano, T.; Toyoshima, S.; Otera, J. *Chem. Eur. J.* **2001**, *7*, 3321-3327.

(82) Jackson, D. Y. *Synth. Comm.* **1988**, *18*, 337-340.

(83) Sun, C.; Bittman, R. *J. Org. Chem.* **2004**, *69*, 7694-7699.

(84) Cox, R. J.; Gibson, J. S.; Hadfield, A. T. *Chembiochem* **2005**, *6*, 2255-2260.

(85) Chambers, R. D.; Jaouhari, R.; O'Hagan, D. *Tetrahedron* **1989**, *45*, 5101 - 5108.

(86) Patois, C.; Savignac, P. *J. Chem. Soc. Chem. Comm.* **1993**, 1711-1712.

(87) Padmakumari, A. J.; Stille, J. K. *J. Org. Chem.* **1982**, *47*, 468-473.

BIOGRAPHICAL SKETCH

Neil Stowe was born in Atlanta, GA. He went Delta State University in Cleveland, MS where he received his B.S. in Chemistry in 2003. He came to the University of Florida in Fall, 2003. Upon completion of his Ph. D. he plans to work at the Scripps Research Institute in San Diego, California.