REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-02-

0598

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
21-02-2002	Final Report	01-09-2000 to 31-08-2001

4. TITLE AND SUBTITLE
Enhancing the Chemistry Curriculum, Teaching and Research Capabilities by the Implementation of Fourier Transform NMR Spectroscopy.

5a. CONTRACT NUMBER

5b. GRANT NUMBER
F49620-00-1-0353

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Department of the Air Force
Air Force Office of
Scientific Research
801 N. Randolph St.
Arlington, Va., 22203-1977

10. SPONSOR/MONITOR'S ACRONYM(S)
N/A

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
N/A

12. DISTRIBUTION / AVAILABILITY STATEMENT
This document is approved for public release; its distribution is unlimited.

13. SUPPLEMENTARY NOTES
N/A

20020315 047

14. ABSTRACT

The purpose of this grant was to purchase a reconditioned 400 MHz Fourier Transform (FT) NMR instrument. Since the installation and training period, the NMR has been used for a number of courses (Analytical Chemistry, Advanced Inorganic Chemistry, Instrumental Analysis, Student Independent Projects and Undergraduate Research Projects). Laboratory experiments using the NMR have been developed to study and characterize organic and inorganic/organo-metallic chemicals, biochemical metabolites and in undergraduate research investigating new nanotechnology materials. NMR spectroscopy is an essential technique in the undergraduate chemistry curriculum and we plan to expand the use of this instrument in the curriculum and in the individual chemistry lab courses.

15. SUBJECT TERMS
NMR in the Undergraduate Curriculum

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Larry G. Carter
Unclassified	Unclassified	Unclassified	None	21	19b. TELEPHONE NUMBER (include area code) (201) 200-3003

Final Technical Report

The purpose of this grant was to provide funding for the purchase of a reconditioned Bruker AC 300 MHz Fourier Transform (FT) NMR instrument. We have instead purchased a General Electric 400 MHz Omega NMR instrument for $100,000. In addition, New Jersey City University agreed to purchase three Nalorac Z-Spec NMR probes for $25,000 and to pay for the $3,000 in shipping costs. This instrument is a more versatile and has a higher field magnet than the Bruker system we originally quoted. However, this instrument took about one-half year to find and an additional four months to install and train on. Since that time, the NMR is currently being used in a number of courses in chemistry including: analytical chemistry, advanced inorganic chemistry, instrumental analysis, student independent projects, and undergraduate research projects. We are currently developing experiments in organic chemistry and physical chemistry that would use this instrument as well as, more experiments in the aforementioned courses.

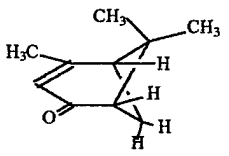
In the experimental descriptions which follow, results from student reports are given showing how this instrument is being used and illustrating the impact that this instrument has on our chemistry curriculum. Students have used this instrument to investigate various chemical problems from studying new materials, food chemistry, natural products, and the characterization of organic and inorganic/organometallic materials to biochemical intermediates and laboratory excercises illustrating NMR concepts. I have also included some research results where the NMR is being used to structurally characterize molecules relevant to molecular electronic components.

Application of the NMR Instrument in the Chemistry Curriculum

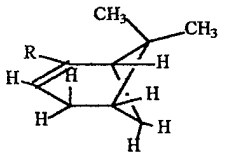
A. Laboratory Experiments in Analytical Chemistry, Instrumental Analysis, Independent Student Projects, and Advanced Inorganic Chemistry.

Experiment 1 - Complete Assignment of Proton Chemical Shifts of Terpenes

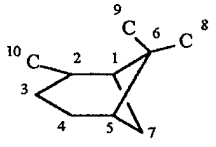
This experiment was run in the instrumental analysis course and served as a good introduction to two-dimensional NMR. Proton nmr spectra of the terpenes: verbenone, myrtenal and α-pinene; were taken. Correlation spectroscopy (COSY) was also investigated by the students to illustrate coupling between protons and to make the interpretation easier.



verbenone



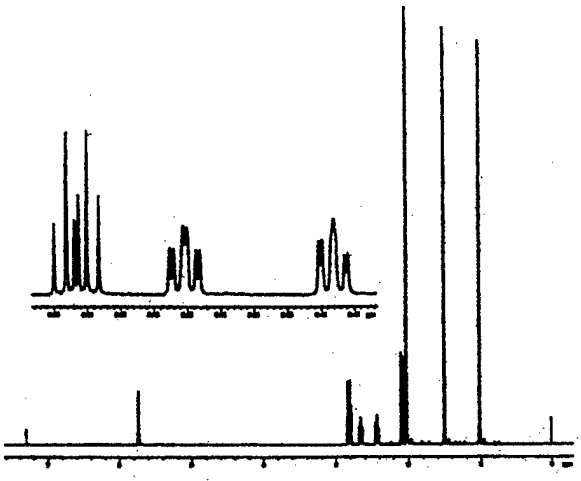
R = CHO, myrtenal
CH_2OH, myrtenol
CH_3, α-pinene



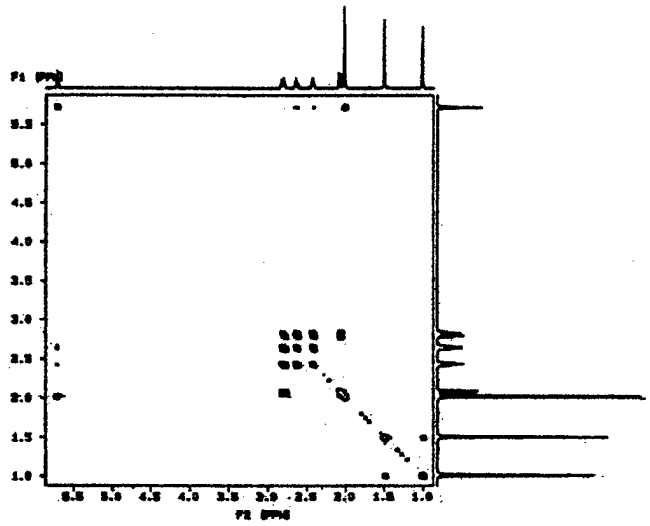
General numbering scheme for NMR spectral peaks

The proton NMR and the COSY spectrum for verbenone are shown below. These were typical spectra taken by students. The assignments were made by choosing protons where the chemical shift assignments were unambiguous; in this case, the proton on the double bond (H-3, $\delta = 5.72$ ppm) was assigned. The COSY spectrum of verbenone showed coupling between H-3 and protons at 2.64 (H-5), 2.43 (H-1), and 2.02 ppm (H-10).

Proton NMR Spectrum of Verbenone



COSY Spectrum of Verbenone



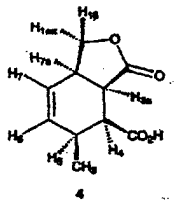
Proton Chemical Shift Assignments for the Terpenes

Compound	H1	H2	H3	H4s	H4a	H5	H7s	H8	H9	H10
Verbenone	2.43	-	5.72	-	-	2.64	2.08	1.51	1.01	2.02
Myrtenal	2.86	-	6.72	2.57	2.57	2.19	2.50	1.34	0.75	9.44
Myrtenol	2.13	-	5.47	2.29	2.26	2.13	2.40	1.29	0.84	3.98
α-Pinene	1.92	-	5.18	2.21	2.17	2.05	2.32	1.23	0.83	1.6

Experiment 2 - The Diels-Alder Reaction of 2,4-Hexadien-1-ol with Maleic Anhydride

The Diels-Alder reaction followed by ring-opening of the cyclic anhydride was performed by students in the organic chemistry lab. NMR structural characterization of the resulting lactone product was accomplished by proton NMR, decoupling experiments and COSY NMR. The results are summarized below.

Proton NMR Assignments for Compound 1

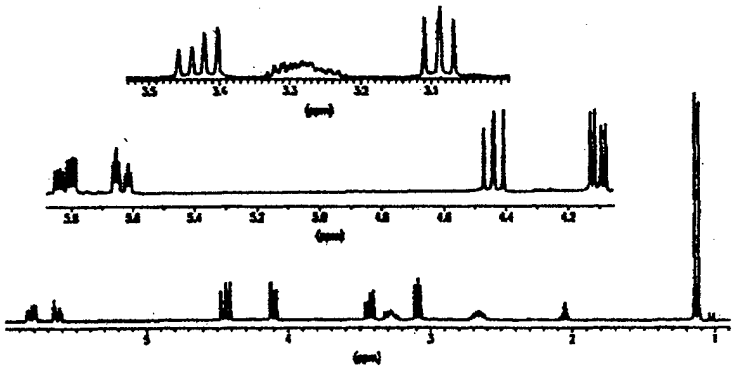


5.81 (ddd, J = 10.1, 4.4, 2.2 Hz, 1 H, H_9)
5.63 (dt, J = 10.0, 2.3 Hz, 1 H, H_7)
4.43 (dd, J = 8.5, 7.4 Hz, 1 H, H_{10})
4.10 (dd, J = 8.5, 3.5 Hz, 1 H, H_{10})
3.38 (dd, J = 9.2, 4.8 Hz, 1 H, H_{5a})
3.28 (m, 1 H, H_{7a})
3.08 (dd, J = 5.6, 4.8 Hz, 1 H, H_4)
2.65 (m, 1 H, H_5)
1.12 (d, J = 7.3 Hz, 3 H, $-CH_3$)

The proton spectra shown below show no overlapping resonances and allows students to calculate coupling constants (J values) and chemical shifts. The spectrum contains four sets of doublet of doublets, a doublet of triplets (δ = 5.63), and a doublet of doublet of doublets (δ = 5.81). Through a series of decoupling experiments, proton peak assignments are made. The COSY spectrum allows students to determine the connectivity of the molecule and tracing the

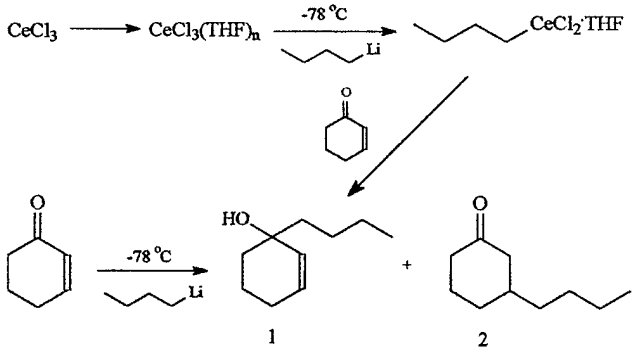
coupling pattern of the off-diagonal elements serves to illustrate to students the correlation of molecular structure to various NMR spectroscopic data. In general, students were very impressed with the power and capability of NMR to elucidate the structure of molecules. Based on these experiments, students were able to make the following assignments for compound 1.

Proton NMR Spectrum of Compound 1



Experiment 3 - Mechanistic Study of an Epoxide Grignard Reaction

The products 1 and 2 can be formed depending on the order in which reagents are added. The proton and carbon spectra are used to verify product formation. The methylene protons in product 2 appeared as a doublet at 2.9 ppm and the methine protons appear as a triplet at 4.6 ppm. The carbon and proton assignments are given below.



Product 1: ^{1}H NMR ($CDCl_3$): δ 5.81 (m, 1H), 5.62 (br. d, 1H), 1.98 (m, 2H), 1.75-1.25 (m, 11H), 0.91 (t, 3H)
^{13}C NMR ($CDCl_3$): δ 132.8, 129.8, 129.6, 69.6, 41.99, 35.31, 25.65, 25.17, 23.22, 28.98, 14.03
Product 2: ^{1}H NMR ($CDCl_3$): δ 2.48-2.20 (m, 2H), 2.08-1.02 (m, 13H), 0.90 (t, 3H)
^{13}C NMR ($CDCl_3$): δ 199.1, 48.16, 41.43, 39.01, 36.23, 31.27, 28.79, 25.23, 22.65, 13.95.

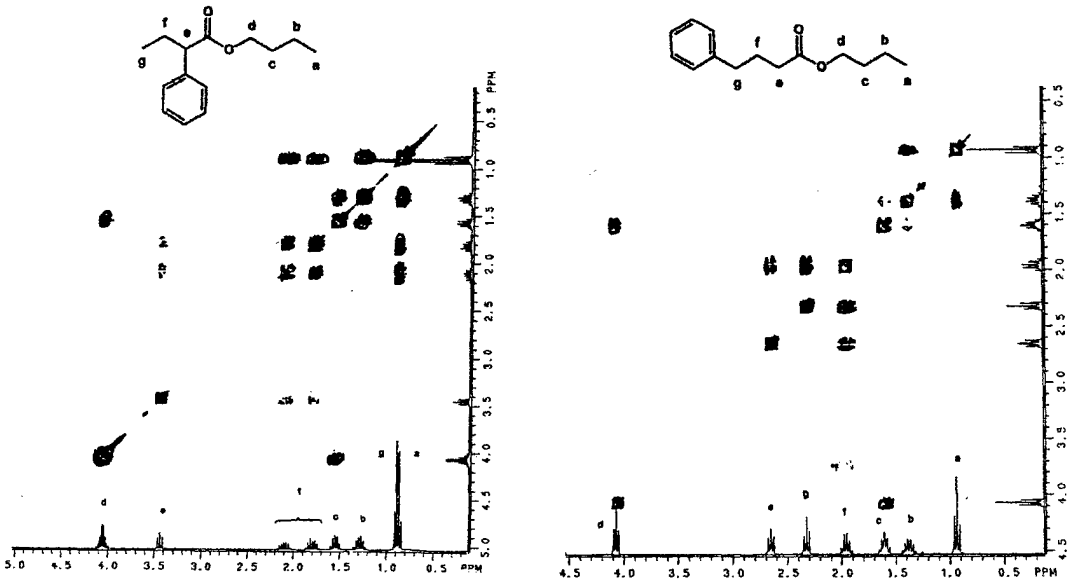
Experiment 4 - Sharpless Asymmetric Dihdroxylation.

This experiment is currently being performed by an independent studies student to verify the results of this experiment. If the stereochemical dihydroxylation product is formed with osmium-chiral amine catalyst, the experiment will be incorporated into the Instrumental Analysis course. Asymmetric catalysis of organic substrates is an important concept in organic chemistry

and experiments that illustrate this concept must be incorporated into the modern chemistry curriculum. The experimental protocol is given in Appendix 1.

Experiment 5 –Microscale Preparation and COSY Analysis of an Unknown Ester.

Students synthesized various unknown aromatic esters from four-carbon alcohols with unknown aromatic carboxylic acids. The diastereotopic esters gave rise to distinct NMR resonances from the proton and carbon spectra. The COSY spectra was used to determine unambiguous proton coupling. Two of these student results are shown for butyl 2-phenylbutyrate and butyl 4-phenylbutyrate illustrating the utility of NMR in determining structures of organic molecules. A copy of the experimental protocol is given in Appendix 2.



Experiment 6 – Synthesis and Characterization of the Dihydrogen Complex, trans-{(Fe(η^2-H_2)(H)[1,2-bis(diphenylphosphino)ethane]$_2$}[BF_4]

The title complex was prepared by students by a protonation reaction of the dihydride complex.

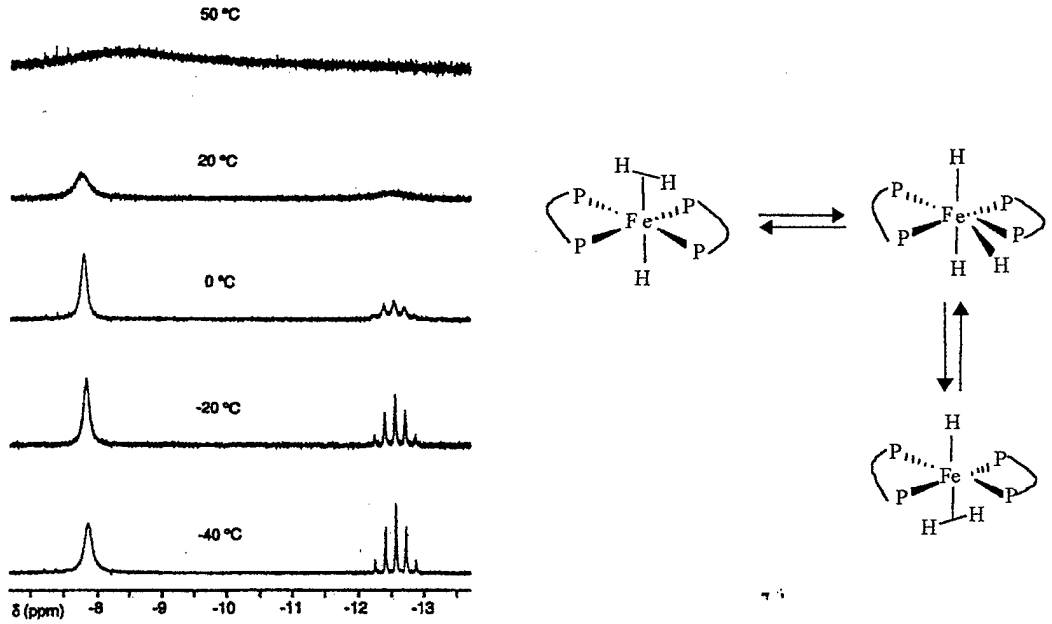
$$\text{cis-Fe(H)}_2\text{(dppe)}_2 + H^+ \longrightarrow \underset{\underline{1}}{\text{trans-[(Fe}(\eta^2\text{-H}_2)\text{(H)(dppe)}_2^+}$$

^{1}H and ^{31}P NMR spectra were recorded for the iron hydride complex shown below

	NMR Data	
Complex	^{31}P {^{1}H}	^{1}H
cis-Fe(H)$_2$(dppe)$_2$	90.8, 101.8	- 13.1 (m)
trans-[(Fe(η^2-H$_2$)(H)(dppe)$_2$][BF_4]	92.5	-12.54 (quintet, 1H) -7.85 (H_2, br. singlet)
Fe(H)(dppe)($C_6H_4PPhCH_2CH_2PPh_2$)	23.7(C_6H_4P, m), 95-103(Ph_2P, m)	- 14.2 (m)

Variable-temperature ^{1}H NMR spectra were also recorded by students shown below for the hydride region of compound 1. The room temperature spectrum had two broad singlets at δ -7.9

and –12.6 ppm, respectively. At low temperatures (-20 and –40 °C), the molecule **1** is in the slow-exchange regime. Also, a quintet is observed corresponding to the hydride resonance, due to the coupling to four equivalent phosphorus nuclei. This temperature dependence is due to the fluxional process in which the hydrogen atoms of the hydride and dihydrogen ligand exchange sites.

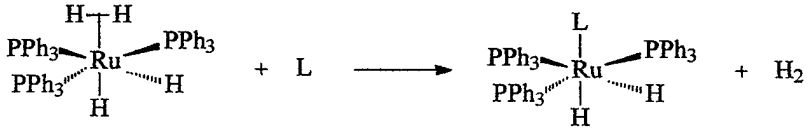


Experiment 7 – Micropreparation and Characterization of $[RuH_2\{PPh_3)_4]$

Students synthesized the above compound, $[RuH_2(N_2)(PPh_3)_3]$ and $[RuH_2(CO)(PPh_3)_3]$ by the following reactions and characterized the products by ^1H and ^{31}P NMR spectroscopy.

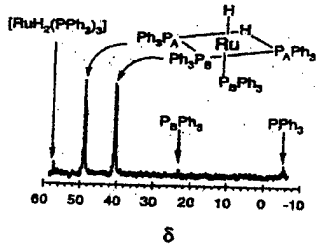
$$RuCl_3 + 3\,PPh_3 + CH_3OH \longrightarrow [RuCl_2(PPh_3)_3] + HCHO + HCl$$

$$[RuCl_2(PPh_3)_3] + 2\,BH_4^- + PPh_3 \longrightarrow [RuH_2(PPh_3)_4] + 2\,B(OCH_3)_3 + 3\,H_2$$



L = CO, N_2, PPh_3

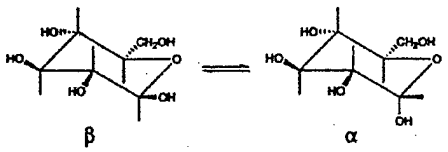
The stereochemical arrangement of the phosphine ligands around the ruthenium metal center creates different ^{31}P chemical shifts. In the phosphorus NMR spectrum, students observed the phosphorus ligand trans to the other phosphorus ligand has a different chemical shift than the phosphorus ligand that is trans to the hydride ligand.



This experiment was well received by students and involves air-sensitive techniques and more sophisticated synthetic procedures and instrumentation. The success of this experiment has prompted us to develop more organometallic experiments that utilize sophisticated instrumentation.

Experiment 8 – Following Glycolysis using ^{13}C NMR

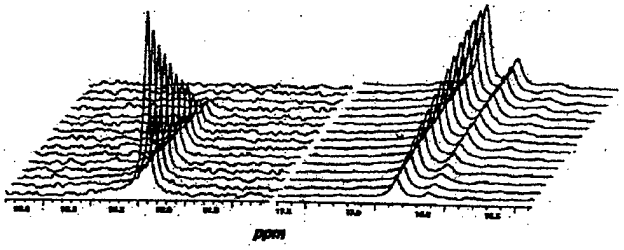
Intermediate metabolites and products of the anaerobic glycolytic pathway were followed by ^{13}C NMR. The ^{13}C peaks for the α– and β-glucose anomers (δ 96 and 92 ppm) were observed by students. This is the only direct method for students to observe phenomena of this type that we frequently discuss in lecture.



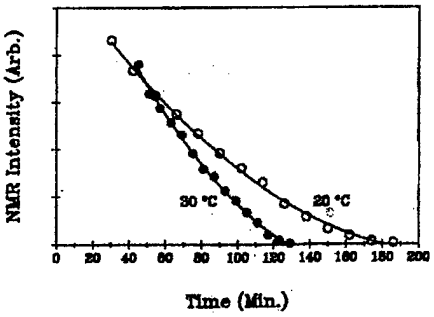
β α

These signals decreased with time during anaerobic glycolysis while signals corresponding to ethanol (δ 57.3 and 16.8 ppm) increased in intensity. This following stack NMR spectrum shows clearly the disappearence of the glucose anomers and the formation of the ethanol with time. Students plotted this data as a function of temperature and observed an increase in the reaction rate, however the kinetics are complicated and students were not asked to determine rate constants.

Stack Proton-decoupled ^{13}C NMR

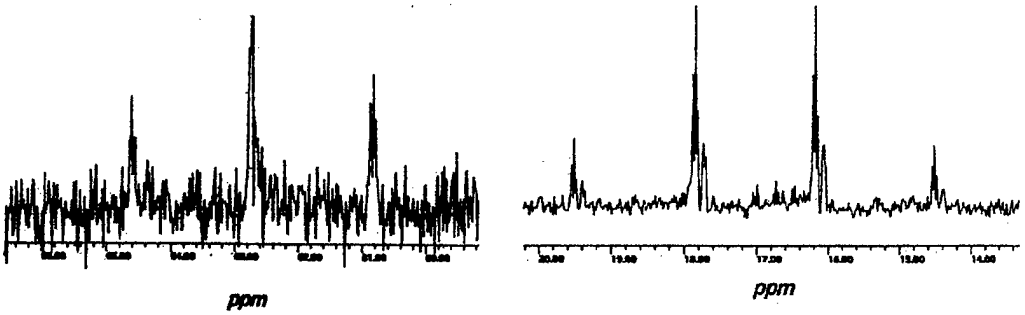


Plot of peak height (δ = 92 ppm) versus time (min) for α-[1-^{13}C]glucose: (○) 20 °C; (●) 30 °C.



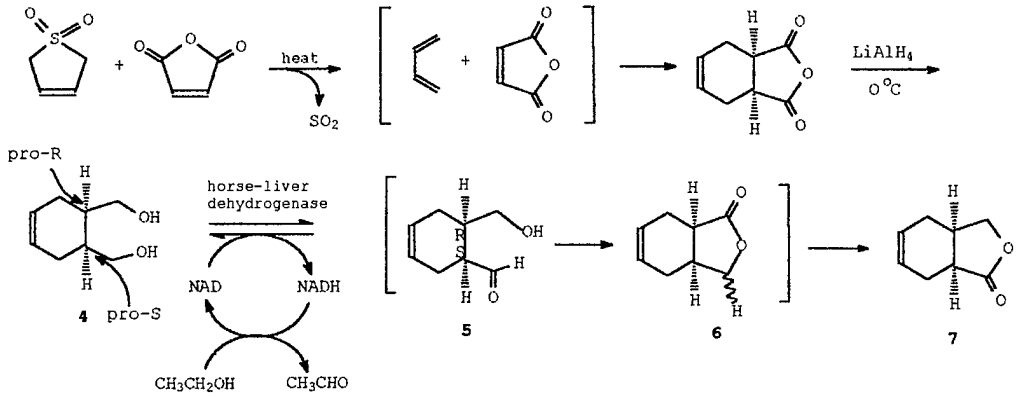
After complete digestion from the anaerobic glycolysis process, the final product proton-coupled carbon-13 spectrum had resonances clearly due to the formation of ethanol.

Proton-coupled ^{13}C NMR Spectrum of the Final Product of Anaerobic Glycolysis by Yeast



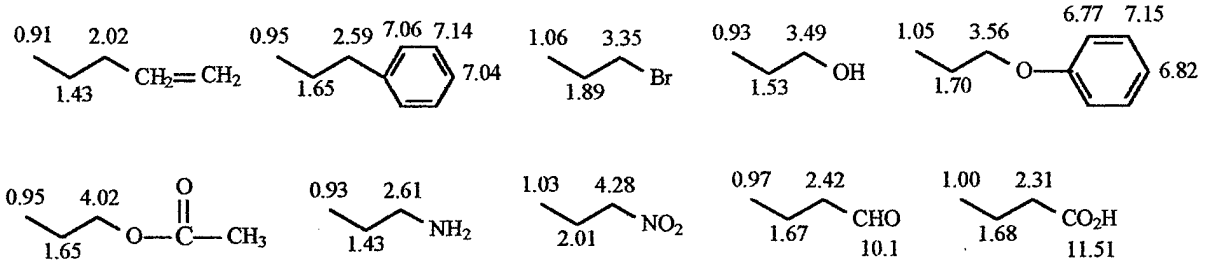
Experiment 9 – Chemoenzymatic Synthesis of an Enantiomerically Pure Lactone

This experiment will be run by students this semester and exemplifies the high stereo-specific nature of biochemical reactions. Horse-liver alcohol dehydrogenase will be used to oxidize the diol 4 to the aldehyde (5) which cyclizes to the lactone, 7. This lactone product will be characterized by proton, carbon, COSY, and HETCOR NMR spectroscopy. The experimental protocol is included in Appendix 3.



Experiment 10 – Comparing Predicted and Experimentally Derived NMR Chemical Shifts.

Students interpreted factors that affect chemical shifts by investigating chemical shift values for a series of related propyl compounds with electron-withdrawing or –donating groups. The following chemical shift values were generated experimentally by students and compared to equations used to calculate these values. The experimental protocol is given in Appendix 4.



B. Undergraduate Research

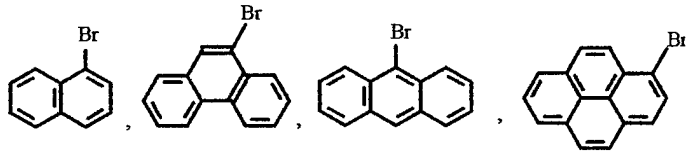
One of the areas of research interest for Dr. Yamaguchi is the study of inorganic coordination compounds and metal-containing dendrimers as components in photo- and electroactive small electrical circuits. The ability to use molecules in small electrical circuits could potentially result in devices with low energy requirements, high speed of operation, and low manufacturing costs. This project involves the synthesis and characterization of several key intermediates and products by NMR spectroscopy.

In general, molecular-scale communication components and devices must contain at least three components: i) a donor group; ii) an acceptor group; and iii) a bridge unit. This research project involves synthetically attaching several types of donor groups to ethynyl- and diethynyl-bridging groups that are attached to different acceptor molecules. Metal ions (Zn^{2+}, Cu^{2+}, Ni^{2+} and Al^{3+}) will be chelated to these acceptor groups. These compounds and its components are characterized by UV-visible, fluorescence, NMR, IR, and LC-MS). In the discussion that

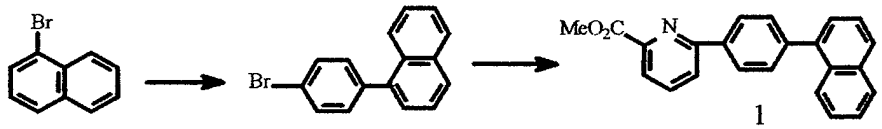
follows, the NMR results are presented for compounds that have been synthesized or are currently being synthesized. The NMR instrument has been key to the identification of these molecules and because much work on this project remains to be completed, NMR spectroscopy will play a more important role to the success of this project.

We are investigating inorganic coordination compounds containing metal ions (Al^{3+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, and Cu^{+}) bound to carboxylate and phenolate chelating groups of 8-hydroxy-quinolate, picolinate, salicylate, and benzimidazoyl, ligands. These will be covalently connected to fluorophoric groups (Scheme 1) and in some cases, to ethynyl and diethynyl acetylenic bridging molecules appended to dendrimer wedges.

Scheme 1



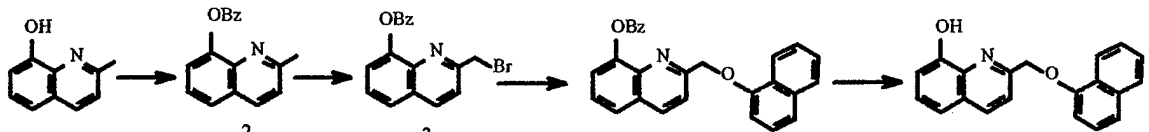
Palladium-catalyzed Suzuki and Heck coupling reactions and the tin-catalyzed Stille coupling reactions have found great utility in organic chemistry and is used in this project for carbon-carbon bond coupling reactions between aryl and ethynyl groups. The fluorophore units are appended to carboxylic acid, phenol and nitrogen containing ligands to increase the luminescence properties of metal complexes. Ligand 1 is currently being synthesized via a Suzuki coupling reaction and will serve as a model for fluorescence enhancement using picolinate ligands. This napthalyl attachment group will in turn be coupled to protected 8-quinolinate ligands and chelated to Al^{3+}, Cu^{2+}, Zn^{2+}, and Ni^{2+} metal ions (after a deprotection step) and the photophysical properties of the metal complex investigated.



1

Synthesis of 2-(Naphthalen-1-yloxymethyl)-quinolin-8-ol (7)

Recently, we have appended a napthyl fluorophores onto an 8-quinolinol ligand using the synthetic strategy shown below. Metal complexes with 8-hydroxyquinoline have been shown to exhibit fluorescence and alkyl-, sulfonic acid-, and amido-substituted quinolinato moiety were shown to have an effect on the luminescent properties of the molecules.



2 3 4 5

The phenol functionality was protected by reaction with benzoyl chloride. In retrospect, we will protect this group using chloromethyl methyl ether (MOMCl), dimethylsulfate, or t-butyldimethyl silyl chloride (TBMS) because of the difficulties in removing the benzyl alcohol after the final reductive cleavage. The phenyl ester (5) was chromatographed and identified by IR and ^{1}H NMR. A Wohl-Ziegler free radical bromination method produced 6 and reaction with 1-naphthol, the naphthyl derivative. Both products were analyzed by HPLC, IR and NMR and were consistent with what was expected for the products. Deprotection of the naphthyl-appended

ligand by reductive cleavage of the phenyl ester moiety from the parent qunolin-8-ol resulted in the desired ligand (7) in high purity by HPLC. Signature for the loss of the benzoyl group was the absence of the carboxyl peak at 1745 cm^{-1} and the large OH stretch at 3396 cm^{-1} in the FTIR spectrum. The methylene peak in the proton NMR spectrum was observed at 4.65 ppm and the proton-decoupled ^{13}C NMR spectrum contained the correct number of peaks expected for the aromatic region of the desired compound. The spectroscopic results are summarized below.

Benzoic acid 2-methyl-quinolin-8-yl ester (2): Yield (97%). ^1H NMR: δ(ppm) = 2.72(s, 1H); 7.37(d, 1H); 7.58(m, 1H); 7.67(m, 1H); 8.20(d, 1H); 8.38(d, 1H). IR: ν (cm^{-1}) = 3092, 2923, 1745, 1602, 1503, 1262, 1223, 1094, 1023, 748 (br.).

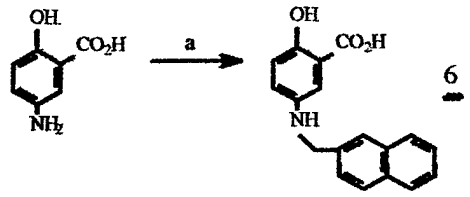
Benzoic acid 2-bromomethyl-quinolin-8-yl ester (3): Yield (72%); ^1H NMR: δ(ppm) = 2.49 (s, methyl); 4.43(s, methylene); 7.02(d, 1H); 7.44(m, 5H); 7.87(t, 1H); 7.98(d, 1H); 8.27(m, 1H). IR: ν (cm^{-1}) = 3064, 1744, 1601, 1503, 1466, 1454, 1431,1314, 1262, 1226, 1176, 1090, 1022, 860.

Benzoic acid 2-(naphthalen-1-yloxymethyl-quinolin-8-yl ester (4): ^1H NMR: δ(ppm) = 4.43(s); 5.01(s, OH from 1-naphthol); 6.45(d); 7.05(m); 7.17(m); 7.40(m)7.64(d); 7.83(m); 8.20(d); 8.33(m). IR: ν (cm^{-1}) = 3055, 1745, 1597, 1579, 1548, 1515, 1452(w), 1386, 1263, 1091, 1042, 1022, 978(w), 770(br).

2-(Naphthalen-1-yloxymethyl)-quinolin-8-ol (5): ^1H NMR: δ(ppm) = 4.63(d, 2H); 6.75(d, 2H); 7.21(m, 4H); 7.39(d, 2H); 7.45(m, 3H); 7.53(m, 1H); 7.60(d,2H); 7.83(d, 2H); 8.22(d, 2H); 8.40(d, 2H). ^{13}C NMR (partial proton-decoupled) δ(ppm) = 109.4; 118.1; 120.3; 121.5; 122.0; 124.8; 125.6; 126.0;126.4; 126.8; 127.7; 128.1; 128.3; 128.6; 129.5; 130.7; 133.8; 134.5; 152.0. IR: ν (cm^{-1}) = 3396(OH), 3053, 1714, 1574, 1505, 1455, 1385, 1274, 1093, 1058, 878, 792.

Synthesis and Characterization of 4-[(Napththalen-2-ylmethyl)-amino]-salicylic acid (6)

This ligand was synthesized to demonstrate that: i) an intramolecular mechanism does not inhibit the PET process of attached fluorophore (naphthyl) group by electronic interaction with a proximal NH group; and also, ii) a chelating carboxylate group is capable of supporting intramolecular electronic interaction and does not quench the fluorescence. It is known in some cases that proximal amino group to a fluorophores can cause fluorescence quenching by electronic interaction with the nonbonding pair of electrons and that in iminodiacetic acid containing bipyridyl ruthenium(II) complexes, the non-chelated but deprotonated carboxylate groups increases the emission intensity of the triplet MLCT state. In a series of gallium(III)-8-hydroxyquinolate and quinaldine complexes,[34] a bound benzoate group exhibited high light output currents while other carboxylate ligands had currents comparable to the aluminum-8-quinolate complexes. To test this hypotheses, the title compound (32) was prepared by a standard alkylation reaction using 2-bromomethyl naphthylene.



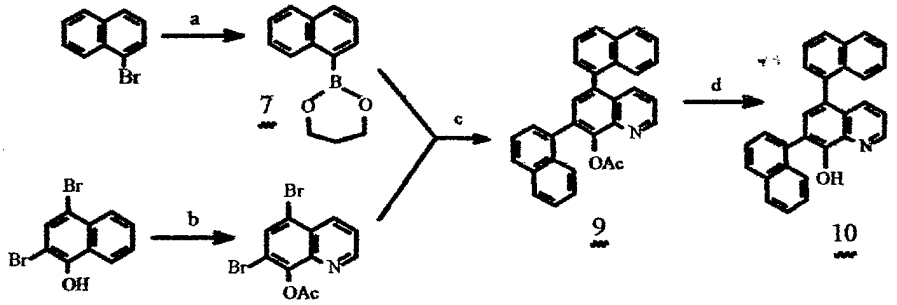
6

The yield was 85% and chromatographed to a single peak. The proton NMR spectrum contained the desired 4.61 ppm methylene peak which integrated to a 2:9 ratio when compared to the napthyl proton signals. No significant shifts in the carbonyl stretching frequency occurred in the IR spectrum, indicating that the 2-bromomethyl naphthalene did not esterfy the carboxylate group and a large OH peak showed that the ether did not form. The spectroscopic data is shown below.

4-[(Naphthalen-2-ylmethyl)amino] salicyclic acid (6): Yield (85%); ^{1}H NMR: δ(ppm) = 4.61 (s, methylene); 5.44(s,1 H); 6.33(m, 2H); 7.47(d, 2H); 7.55(m, 3H); 7.61(d, 2H); 7.85(m, 1H), 7.99(s, 2H). IR: ν (cm^{-1}) = 3416 (OH), 3215(NH), 2894, 2676, 2487, 1633 (C=O), 1547, 1510,1304, 1275, 1155, 826. UV-Vis: MeOH, λ_{max}(nm)= 298(sh), 292, 281, 276(sh), 260.

Synthesis of 5,7-bis(napthyl)-8-O-actetyl quinoline (10)

We have very recently started on the 1-(trimethyleneborate)-naphthylene as a key precursor to appending fluorophores onto ligands. The 1-(trimethyleneborate)-naphthylene (7) and the acylated 5,7-dibromo-8-O-acetylquinoline (8) products were synthesized in order to carry out the Suzuki coupling reaction. The synthesis of the dinapthyl product (9) is currently being worked on before the deprotection step, yielding the desired ligand, 10. This ligand will be chelated to the above mentioned metal ions and fluorescence measurements made.

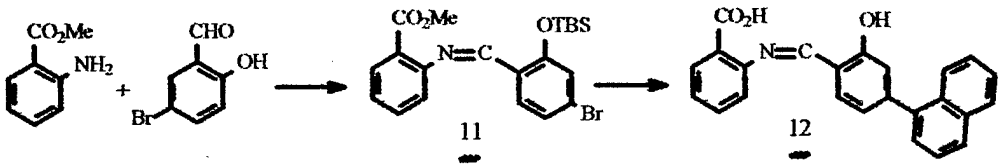


1-(trimethyleneborate)-naphthylene (7) . ^{1}H NMR: acetone d_6 δ(ppm) = 1.95 (quin, 2 H); 4.08(t,4 H); 7.55(m, 3H); 7.33(m, 4H). ^{13}C NMR: acetone d_6 δ(ppm) = 61.95(middle CH_2 in alkylated borate); 100.41(outer two methylene in alkylated borate); 124.87, 125.09; 125.81; 127.76; 128.27; 128.32; 130.80;. 133.30; 134.24; 136.63. IR: ν (cm^{-1}) = 3040, 1948, 1823, 1726, 1595, 1573, 1480, 1434,1416, 1322, 1302, 1271, 1251, 1207, 1153, 1132, 1087, 1019, 967.

O-acetyl-5,7-dibromo-8-hydroxyquinoline (8). $CDCl_3$ 1H NMR: δ(ppm) = 2.25 (s, 3H); 7.13(m, 1H); 7.61(t, 1H); 8.12(m, 1H); 8.55(s, 1H). ^{13}C NMR: $CDCl_3$ δ(ppm) = 21.76(CH_3); 100.10; 105.33, 116.35; 118.92; 122.50; 132.76; 135.45; 141.98;. 145.04; 151.32; 167.89. IR: ν (cm^{-1}) = 2931, 2680, 1764, 1582, 1474, 1457, 1431, 1364, 1184, 1129, 1037, 1007, 929, 884, 854, 784, 691.

Synthesis of N-(2-Carboxyphenyl)-4-bromosalicyladimine (12)

This ligand was recently synthesized by condensing anthranilic acid (o-aminobenzoic acid) with 5-bromosalicyaldehyde. The proton and carbon NMR are consistent with the synthesis of this product; however, the product is impure containing a substantial amount of starting materials.



6-bromo-methylpicolinate (11). $CDCl_3$ 1H NMR: δ(ppm) = 8.10(m, 1H), 7.70(m, 2H), 4.02 (s, 3H). ^{13}C NMR: $CDCl_3$ δ(ppm) = 139.25, 131.86, 124.08, 53.19, 7

NMR Spectroscopy in the Undergraduate Chemistry Curriculum at New Jersey City University.

A summary of student experiments and research is described using the recently purchased NMR. It is apparent that the type of experiments that can be done with this instrument will dramatically improve the chemistry curriculum. This FT-NMR instrument complements other recently acquired analytical instruments (laser Raman, LC-MS, scanning fluorimeter, capillary electrophoresis, etc.) and will allow students to get involved in every aspect of studying chemical reactions and structural. The GE 400 MHz NMR instrument is capable of acquiring ^{1}H and ^{13}C spectra and able to perform pulse-sequence techniques (COSY, HETCOR, NOESY, ROESY, etc.). Experiments have been developed in the analytical, instrumental analysis, inorganic, advanced organic, physical and biochemistry courses; too given students a range of experience using this important technique. Students obtain substantial experience not only with routine applications for structural determination, but also with advanced applications with pulse-sequencing techniques and studying temperature-dependent reactions. In the future, other and more extensive student experiments will be developed using NMR to structurally characterize a variety of organic, inorganic, organometallic, polymers and new materials. In addition, NMR will become increasingly more important to undergraduate student research projects. This NMR instrument is an irreplaceable component for the spectroscopic identification and characterization of molecular systems in the chemistry curriculum.

Appendix 1

Sharpless Asymmetric Dihydroxylation:

Sharples's methods for the symmetric oxidation of alkenes, namely, asymmetric epoxidation, (AE) and asymmetric dihydroxylation (AD), stand out because of their wide utility and widespread usage. The experiment described here is an investigation into the effect of alkene structure on Sharpless AD.

Asymmetric Dihydroxylation

The diastereospecific cis-dihydroxylation of alkenes with osmiym tetroxide is a useful procedure for the introduction of oxygen-containing functionality into a molecule in a stereo-defined fashion. The second oxidant effects oxidative hydrolysis of the initially formed cyclic osmate ester in situ, thereby liberating the cis-diol concomitant with regeneration of the osmium tetroxide to complete the catalytic cycle. The Sharpless procedure employs catalytic quantities of osmium tetroxide and single-enantiomer chiral amine ligands in conjunction with potassium ferricyanide and potassium carbonate [$K_3Fe(CN)_6$-K_2CO_3] as stoichiometry secondary oxidant in a biphasic solvent medium. (Fig.1)

Coordination of the chiral amine ligand to the osmium leads to the formation of a chiral complex. This complex and distinguish between the prochiral faces of a substrate alkene, resulting in cis-diol formation on one face of alkene in preference to the other. The degree of enantiometric enrichment reflects the extent to which the complex can distinguish the two prochiral faces of the alkene. This is dictated by the difference in energy between the two diastereomeric transition states leading to reaction on either face and is determined by a combination of (I) the structure of the chiral amine-containing osmate complex and (ii) the structure of the alkene substance. Opposite enantiomers of the chiral amine give opposite enantiomers of cis-diol products.

Sharpless has designed a chiral ligand system consisting of two naturally derived dihydroquinine (DHQD) alkaloid units linked together by a phthalazine(PHAL) linker [$(DHQD)_2PHAL$, Fig 2). The enantiomeric alkaloid does not occur in nature, so he had to use the naturally derived diastereomeric dihydroquinidine- (DHQ-) based analogue for this purpose I (DHQ_2PHAL) To all intents and purposes these two ligands behave as if it crucial to the enantio-differentiating event in cis-dihydroxilation and the difference in configuring at the quinuclidinic ethyl substituent is not important.

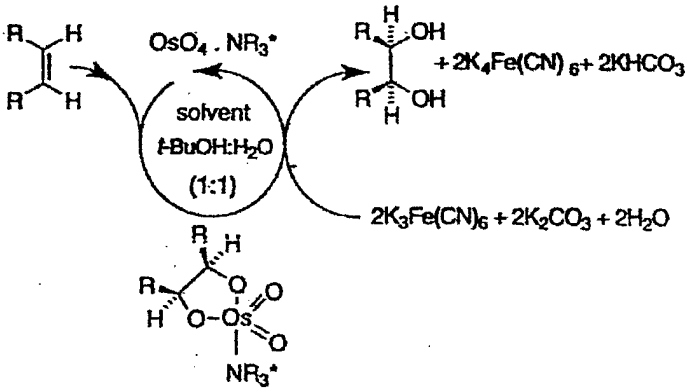
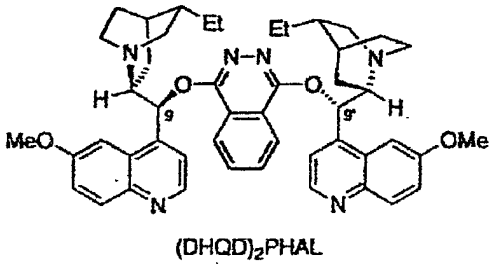
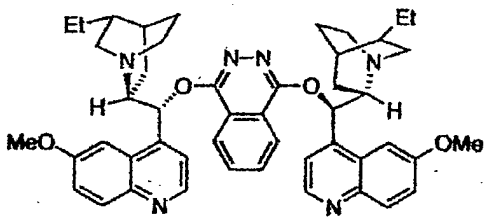


Figure 1. Catalytic cycle for Sharpless asymmetric dihydroxylation. For example, NR_3^* = $(DHQD)_2PHAL$ (single enantiomer chiral ligand



$(DHQD)_2PHAL$



$(DHQ)_2PHAL$

Figure 2. Chiral ligands for Sharpless asymmetric dihydroxylation.

Using the above phthalazine-based ligand systems, moderate to good levels of enantioselectivity can be achieved for most alkene. That so many different types of alkenes can be efficiently dihydroxilated with high enentioselectivity is a particularly attractive feature of the AD process. Such broad substrate tolerance is rare in symmetric catalysis.

The quasi-enantiomeric ligand systems ($[DHQD]_2PHAL$ and $[DHQ]_2PHAL$) and are commercially available as constituents of ready-mixed oxidizing systems known as "AD mix B", which contains $(DHQD)_2PHAL$, and "AD mix a", which contains $(DHQD)_2PHAL$.

Sharpless has provided a model for predicting the sense of enantioselectivity of dihydroxylation of a particular type of alkene with these ligands and this most easily presented in the form of a pictorial mnemonic (R_S, R_M and R_L refer to "small", "medium" "large" substances respectively, Fig 3).

The experiment

This experiment involves the six monitored by TLC at 30-min intervals. The time required for each reaction completion varies between 2 and 20 hrs. (see experimental section). Although the data are only qualitative, reliable relative rate data can be inferred from the time taken for the alkene to be consumed provided that the amount of osmium added is strictly constant in all the reactions. We have found that this can be achieved by the use of accurately pre-weighed vials of AD mixes and osmate salt.

All three cis-diol samples are subjected to chiral GC or HPLC analysis to determine the purity and level of enantio-selection achieved in the AD reactions.

Figure 3 - Sharpless's Mnemonic for Predicting Sense of Enetioselection in AD Reaction

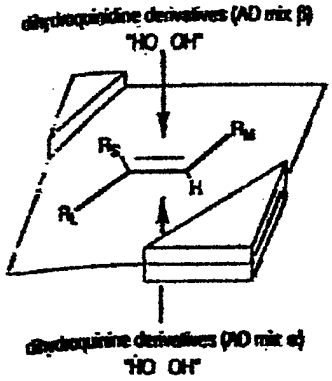
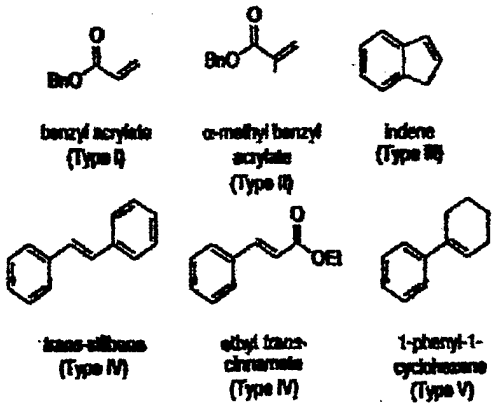


Figure 4 - Alkenes for this Experiment



To AD mix B (1.4g) in a 50-mL round-bottom flask containing a stir bar was added distilled water (5-mL) and tert-butanol (5-mL). The resulting suspension was stirred (and if necessary warmed gently with a hair dryer) until two clear phases were obtained; the lower aqueous phase appeared yellow. Stirring was maintained during dropwise addition of styrene (C_8H_8, MW = 104, d = 0.91, 114 μL, 1.0 mmol) by microliter syringe. The resultant heterogeneous slurry was stirred vigorously in the dark at room temperature until the reaction was complete by TLC (ca. 4 hrs). Sodium metabisulfite ($Na_2S_2O_5$, 0.5g) was added slowly to the reaction mixture, followed by water (20 mL), and the resulting suspension was stirred at room temperature for 30 min. The reaction mixture was then transferred into a 100 mL separatory funnel with two portions of chloroform (2 x 20 mL). The lower organic phase was separated off and aqueous phase was re-extracted three times with chloroform (3 x 20 mL). The combined organic phases were dried over sodium sulfate ($Na_2S_2O_4$) and filtered into 100 mL round bottom flask (washing the sodium sulfate twice with small portions of chloroform), and the filtrate was evaporated to leave a pale yellow oil (110 mg, 80%).

Notes

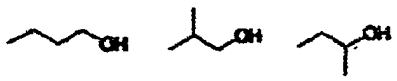
1. No type VI tetrasubstituted alkene was provided. Initially we plan to use 3,4-dihydro-2-methyl-1-(tert-butyldimethylsilyloxy)-naphthalene as a type VI alkene (tetraalkyl alkenes react too slowly). However, its preparation on a large scale proved troublesome and since the product is an alphahydroxy ketone (19), comparison of its rate of reaction with the other alkenes is not straightforward.
2. Clean reaction flasks are essential to avoid accidental inclusion of materials known to bring about the rapid decomposition of high energy oxidants. (e.g., traces of materials).
3. During asymmetric dihydroxilation, reaction flasks should be wrapped in aluminum foil to protect from direct sunlight, which destroys the catalyst.
4. Some ester hydrolysis occurs during workup of the substrate with aqueous sodium sulfite. Sodium metabisulfite should be used instead.
5. This substrate is thermally unstable and should be kept in the refrigerator.

Appendix 2

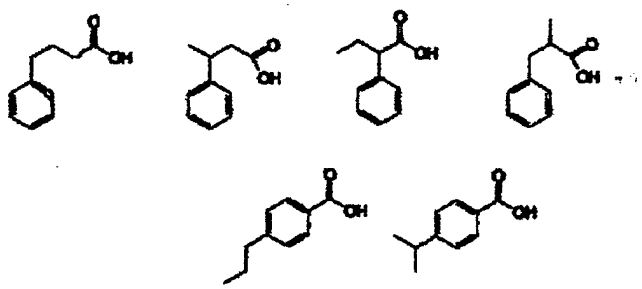
Characterization of Aromatic Esters by 2D-NMR, IR, and GC Analysis of Products

In this experiment, esterification of a series of $C_4H_{10}O$ alcohols and $C_{10}H_{12}O_2$ aromatic carboxylic acids are studied. All possible combinations of the alcohol and acids are investigated using an acidic ion-exchange resin to catalyze the esterification reaction. The three alcohols and five aromatic acids are shown below. The acids correspond to: 4-isopropyl benzoic acid, 2-phenyl butyric acid, 3-phenyl butyric acid, and 4-phenyl butyric acid. The alcohols are: 1-butanol, 2-methyl-1-propanol (isobutanol), and 2-butanol. When esterified with a chiral acid, both n-butyl alcohol and isobutyl alcohol have diastereotopic groups that may give rise to distinct resonances in the NMR spectrum.

The $C_4H_{10}O$ alcohols.



The $C_{10}H_{12}O_2$ acids.



Experimental Procedure

Add 1.5 mmol of the alcohol and 2.0 mmol of the acid into a small vial (~3-5 ml). Add Doxex 50X2-100 acid ion-exchange resin (50 mg). Put a rubber septum on the vial and puncture it with a syringe needle to vent while refluxing. Heat the mixture for 1 hr and allow to cool to room temperature. Place the mixture in a test tube. Rinse the Dowex resin with small portions of ether (total ~ 6 ml) and add these to the mixture in the test tube. In the test tube add a 3 ml portion of aqueous saturated sodium bicarbonate solution and shake well. Then add saturated sodium chloride (~3 ml) and shake well. Separate the organic phase and add magnesium sulfate to dry the organic layer. Filter the organic layer and record the IR, GC, and ^{1}H-NMR both 1D and 2D (COSY) for the ester. If time allows, record the DEPT –135 and the HETCOR spectra.

Appendix 2

Chemoenzymatic Synthesis of an Enantiomerically Pure Lactone

The use of enzymes as catalysts for organic synthesis is now common and is particularly useful for the preparation of small chiral molecules in enantiomerically pure or enriched form. Dehydrogenase enzymes catalyze oxidations and reductions in which nicotinamide cofactors (NAD, NADH, NADP, NADPH; see Fig. 1) serve as the immediate two-electron oxidants or reductants. These reactions are highly enantioselective, but the cofactors are expensive. In order for preparative-scale to be economically practicable, the cofactor must be regenerated *in situ* to the appropriate oxidation state by a secondary oxidant or reductant, such that only a catalytic amount of cofactor is needed. Thus, a catalytic quantity of nictotinamide cofactor is shuttled back and forth between its oxidized and reduced forms. It serves simply as a two-electron transfer agent; another reagent, the regenerant, acts as the ultimate oxidant or reductant (Fig. 2). The regeneration step may be spontaneous. It may be catalyzed by the same enzyme that catalyzes the synthetic reaction, or it may be catalyzed by a separate enzyme.

This laboratory experiment uses a reaction catalyzed by horse-liver alcohol dehydrogenase (HLADH) to oxidize the *meso diol* **4** as the key step in a synthesis of the enantiomerically Pure lactone **7** (Fig. 3). The enzyme first discriminates between the two enantiotopic groups of the *meso diol*, oxidizing only the pro-S hydroxymethyl group to an aldehyde. The intermediate chiral hydroyaldehyde, **5** then spontaneously cyclizes to form the lactol **6** using the unoxidized (R)-hydroxymethyl group. The lactol **6** is further oxidized by HLADH to the product lactone **7**.

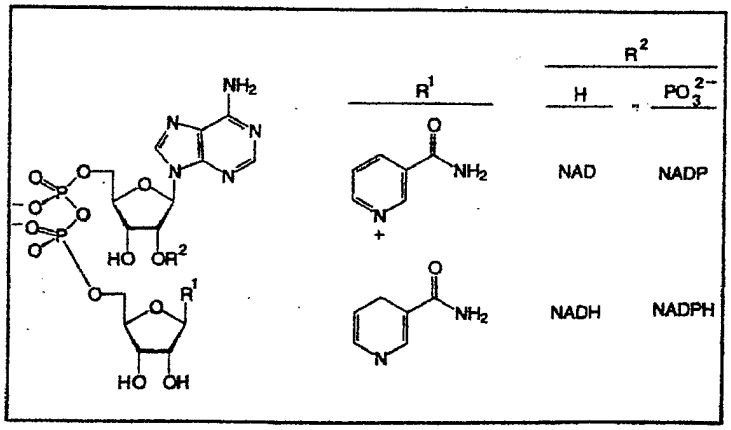


Figure 1. Structures of nicotinamide cofactors NAD, NADH, NADP, and NADPH.

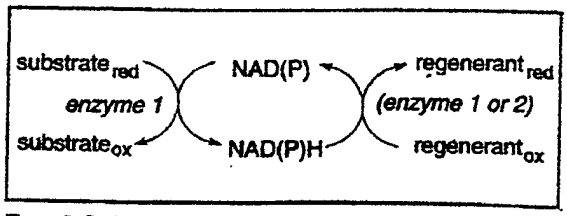


Figure 2. Cycle of a nicotinamide cofactor acting as catalyst.

This series of experiments requires the use of the product from one reaction as the starting material for a subsequent reaction and thus affords experience with a multistep synthesis. In addition, exposure to several important reactions and laboratory techniques is accomplished in this experiment. Thermal extrusion of sulfur dioxide from solfolene is used to generate 1,3-butadiene in situ for a Diels-Alder cyclization. TLC is used during the lithium aluminum hydride ($LiAlH_4$) reduction of the acid anhydride **3** to the diol **4** to follow reaction progress and to note

the formation of the intermediate lactol. The product of the reduction is purified by column chromatography.

The enzymatic oxidation illustrates in situ cofactor regeneration and allows one to measure simple enzyme kinetics. The catalytic activity of the enzyme is determined spectrophotomerically, using Beer's Law. IR spectroscopy is used to observe the changes in carbonyl and hydroxyl stretching frequencies associated with functional group transformations (acid anhydride → diol → lactone).

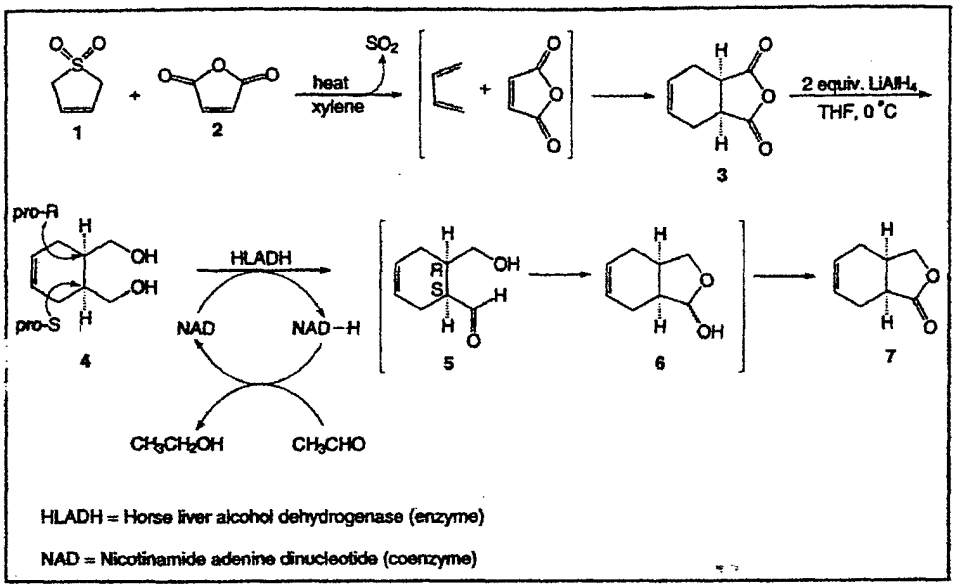


Figure 3. Overall reaction sequence.

Preparation of 3 via Diels-Alder Reaction (5)

The Diels-Alder reaction of 1,3–butadiene is capricious due to the low boiling point of the diene and its tendency to polymerize. However, a tractable source of 1,3-butadiene is 3-sulfolene, 1. Heating 1 to 110-130 °C causes the rapid and complete cycloreversion to sulfur dioxide (a gas) and butadiene. Steady-state generation of 1,3-butadiene in the presence of a dienophile, such as maleic anhydride (2), enables the Diels-Alder reaction between the liberated diene and dienophile to occur cleanly.

Procedure

- ***Caution:*** Sulfur dioxide is very *irritating* to the eyes and respiratory tract; it has a strong, suffocating *odor*.

To a clean 250-mL round-bottom flask are added 8.4 g (0.071 mol) of 3-sulfolene, 1, and 5.0 g (0.050 mol) of maleic anhydride, 2. If the maleic anhydride is in big chunks, pulverize it in a mortar and pestle. Several boiling stones and 3.5 mL of xylene are then added. The flask is tightly stopped to exclude atmospheric moisture and swirled gently for a few minutes. The flask is then put into a heating mantle, and fitted with a condenser and a funnel umbrella (inverted funnel) connected to an aspirator to pull off the SO_2 gas. Heat the reaction for 30 minutes, being careful in the first few minutes because the reaction is exothermic. The aspirator umbrella is left

on during the reflux. After removing the heating mantle, the flask is cooled for only about 5 min. with the aspirator still on. Toluene (50 mL) and 1.0 g of decolorizing charcoal are added.

This suspension is brought to boil and filtered hot through a fluted paper funnel into a dry 250-mL Erlenmeyer flask. A fresh boiling stone is added to the solution, and the filtrate is simmered until the product redissolves. Dry petroleum ether (60-75°C) is then carefully added with swirling until a slight turbidity persists.

The solution is rewarmed until substantially clear, then cooled in ice-water to crystallize the product. If the crystals are oily, the solution can be rewarmed, with a little more temperature before being put into the ice bath. During isolation of the crystals by suction filtration, they are rinsed with a 1:1 mixture of cold petroleum ether and toluene. The crystals can be spread out on a watch glass or in a beaker to dry. Yields ranged from 30-70%, literature melting range, 103-104°C.

Reduction of Anhydride 3 to Diol 4 (7)

Cyclic anhydrides can be reduced to diols by the appropriate choice of reducing agent and solvent. The anhydride **3** is readily reduced under conditions that are milder than those reported for the reductions of other bicyclic anhydrides. Reflux of the reaction is unnecessary. Purification of the diol via chromatography before the enzymatic oxidation, however, is needed.

Procedure

- ***Caution.*** Because this reaction generates hydrogen gas, all flames must be extinguished before any reaction is set up. Students should be admonished not to get $LiALH_4$ on their skin and not to inhale the fine powder when it is weighed out.

Flame-dry a 250-mL round-bottom flask with stir bar using a Bunsen burner, and immediately put a calcium chloride drying tube onto the hot flask. Allow the flask to cool to room temperature. $LiAlH_4$ (approximately 2.0 equiv relative to the anhydride) is then added to the cool flask. Dry THF (stored over molecular sieves, approximately 2.0 mL per mmol of $LiAlH_4$ used) is quickly added to the flask, and the drying tube is attached. The reaction mixture is stirred well and cooled with an ice-water bath. To the stirred suspension, add dropwise a solution of approximately 2 g of the anhydride dissolved in a minimum amount of dry THF. The reaction mixture bubbles vigorously due to evolution of hydrogen gas. When the addition of the anhydride is complete, reattach the drying tube and stir the reaction vigorously. The reaction can be followed by TLC (1:1 ethyl acetate-petroleum ether, iodine visualization) looking for disappearance of the starting material, which streaks up the plate. The reduction is usually complete after 15-30 min. If TLC analysis indicates the presence of starting material after 15 min. at 0°C, remove the ice bath and stir the mixture at room temperature. Continue to monitor the reaction by TLC.

To quench the reaction, first recool the mixture in an ice-water bath. Carefully and slowly add *n* mL of water dropwise and with stirring to the reaction mixture with *n* g of $LiAlH_4$ used. Then, add n mL of 15% NaOH dropwise and with stirring to the reaction. Last, add 3*n* mL of water. The mixture must be kept stirring !. The mixture will get foamy due to the evolution of hydrogen. The ice bath is removed, and the mixture is stirred overnight at room temperature. If stirring overnight is not possible, as soon as a nice granular precipitate is formed, the reaction can be worked up. This may take at least 1 h. The mixture can be left stirring for longer than overnight, but care must be taken to ensure that the reaction does not dry out. The quenched gray $LiAlH_4$ forms a white granular precipitate of aluminum oxide.

The reaction mixture is suction-filtered through a plug consisting of magnesium sulfate on the bottom and Celite on the top; take care to rinse the reaction flask thoroughly. When doing the filtration, the solid cake must not be allowed to dry completely between rinses because a fair amount of the diol sticks to the aluminum waste solids. Continue rinsing the filter cake with THF until no more product is visible by TLC in the THF washes. The solvent is removed from the

filtrate by rotary evaporation, and the crude product is weighed. The crude diol is purified by column chromatography.

Purification of Diol 4

The diol is purified on a silica-gel column, using 50 g of silica gel and 1: 1ethyl acetate-petroleum ether as the eluant. The column can be packed we or dry. The crude diol is dissolved in a minimum amount of the eluant and transferred via pipet onto the top of the column. The first 100 mL of eluant can be discarded. Fractions of 20-30 mL each are collected until the product starts eluting off the column with the higher R_f impurity (follow by TLC). Smaller fractions should then be collected. As soon as fractions containing only the desired material are collected, the polarity of the solvent is increased to 100% ethyl acetate. Elution continues until TLC indicates that no more product is coming off the column. The fractions containing only the product are combined, and the solvent is removed by rotary evaporation to yield a light -yellow oil. Typical yields of the pure diol obtained were approximately 1.2 g (65%).

Enzymatic Oxidation of Diol 4 to Lactone

HLADH catalyzes the enantioselective oxidation of 4 to (–)-(1*S*, 6*R*) – cis-8-oxabicyclo [4.3.0] nonan-7-one, 7. The enzyme first discriminates between the two enantiotopic groups of the meso diol, oxidizing only the pro-S hydroxymethylene group. The intermediate hydroxyaldehyde spontaneously cyclizes to form the lactol 6, which is further oxidized by HLADH to the lactone 7. HLADH requires the cofactor NAD as the hydride acceptor. Because NAD is expensive, regeneration of the cofactor from its reduced form, NADH, is desirable. Acetaldehyde is inexpensive and can be used conveniently as a cosubstrate for the regeneration of NAD from NADH. HLADH catalyzes the NADH-dependent reduction of acetaldehyde to ethanol (eq 1). Thus, NAD can be used catalytically anc regenerated in situ.

$$\frac{\Delta A_{334}}{\text{min}} = \epsilon b \left(\frac{\Delta c}{\text{min}} \right)$$

Procedure

To about 0.5 g of diol 4 in a 500-mL Erlenmeyer flask with a stir bar are added 300 mL of 0.01 M Tris/HCl buffer pH 8.0, 1.0 mL of acetaldehyde, and 10 mL of a solution of NAD in buffer. Tris/HCl buffer is tris (hydroxymethyl) aminomethane hydrochloride. Stir the solution to mix well. The pH of the mixture is checked at this point and readjusted to approximately pH 8 by dropwise addition of 10% NaOH solution. Once the pH is adjusted, add 25 mL of a solution of HLADH. The flask is stoppered and stirred well, but without agitation, frothing, or heating of the solution. The protein may be denatured by heat or mechanical agitation and then precipitate out of solution. If precipitation does occur, reduce the rate of stirring or heat transfer from the stirrer to the flask, and add another 25 mL of the enzyme solution. Stir the reaction overnight.

At the next lab period, check the progress of the reaction by TLC, looking for disappearance of the diol starting material. To prepare a TLC sample, remove about 1 mL of the reaction solution by pipet, and extract by shaking the aliquot in a stoppered vial with about 1 mL of chloroform. Pipet out the chloroform layer and use it to spot the TLC plates. Elute the plates with 30% ethyl acetate in petroleum ether, and visualize in an iodine chamber.

When the reaction looks complete or nearly so, pour the reaction mixture into a separatory funnel and extract the product into hexane (2 x 50 mL). Dry the organic layer over magnesium sulfate. Filter the solution into a large round-bottom flask, and remove the solvent by rotary

evaporation. Weigh the crude lactone. Yields obtained by students were typically around 70%. The specific rotation of the pure lactone is $[\alpha]_D = -67.1°$(*c* 1, $CHCl_3$)

IR Analysis of 3, 4 and 7

The functional-group transformations (acid anhydride → diol → lactone) are readily confirmed by observing the appearance and disappearance of carbonyl and hydroxyl group stretching frequencies in the IR spectra of compounds **3, 4** and **7.** They compare the two spectra with each other and with that of **3**, looking for the differences expected due to changes in the functional groups. Observations are recorded in the students' notebooks.

NMR Spectra of 7

Obtain the proton, proton-decoupled carbon, and COSY spectra for the product. Analyze your results and justify product formation.

Kinetic Assay of HLADH

In this exercise, the students measure the rate of the HLADH-catalyzed oxidation of diol **4** spectrophotometrically by continuous monitoring of the increase in absorbance at 334 nm due to the formation of NADH. Students then calculate the specific activity of the enzyme as units per mg. A unit is a measure of catalytic activity and is the amount of enzyme that will produce 1μmol of product per minute. The exercise requires the use of a UV-vis spectrophotometer.

Procedure

In a 3-mL cuvette are combined 1.5 mL of a prepared solution of diol **4**, and 1.0 mL of a diluted NAD solution. The cuvette is placed in the spectrophotometer and the absorbance at 334 nm is zeroed. The cuvette is removed and 0.50 mL of a diluted enzyme solution is added. The cuvette is quickly covered with a small square of Parafilm and inverted twice to mix the contents. The cuvette is replaced into the spectrophotometer, and the change in absorbance at 334 nm is recorded for about 5 min. The time dependent change in absorbance can be recorded either by use of a strip chart recorder or simply by writing the numerical values of absorbance in a notebook every 30 s.

The rate of change of absorbance, ΔA_{334}/min. is taken from the slope of the line on the strip chart or by least squares fit to the numerical data recorded in a notebook. The rate of change of concentration of NADH (Δc/min) is calculated using Beer's Law

where ε, the molar absorptivity of NADH is 6.18 $mM^{-1}cm^{-1}$ at 334 nm; and *b*, the path length of the cuvette, is 1 cm. Knowing that the total volume of the reaction in the cuvette is 3 mL and knowing the number of milligrams of enzyme added to the reaction, the student can calculate the number of micromoles of product (measured here as NADH) produced per minute mg of enzyme.