INTRODUCTION TO ADVANCED SYNTHESIS
Chem 4330/6330

Lab Manual, 2010 Edition

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GENERAL SYLLABUS

The course Chem 4330/6330 (Advanced Synthesis) in normally taught in the fall semester. This manual provides a general description of the requirements for the course, introduction to the textbook synthetic chemistry, detailed experimental procedures, discussion of specific experimental operations in the laboratory, and discussion of general lab safety. The essentials of synthetic chemistry described in this manual will be elaborated in more detail in the classroom. Numerous references including reviews and monographs are summarized at the end of each particular section. Nonetheless, the following book is strongly recommended as a general guide to laboratory work: T. Leonard, B. Lygo, and G. Procter, Advanced Practical Organic Chemistry, Blackie Academic and Professional, London, 1995, second edition (or equivalent). The complete schedule of the course, the names of Professor, Lab Instructor, and Teaching Assistants, and selection of the experiments for any given semester will be provided at the beginning of the course.

Laboratory Safety

"Wet" laboratories are filled with numerous dangers. It is the responsibility of everyone in the laboratory to look out for safety hazards. Some of the chemicals that you use may be corrosive and carcinogenic, and others may be pyrophoric, potentially explosive, and highly flammable. You can easily access SDBS sheets *via* the internet on computers located in the laboratory to read about any hazards associated with the chemicals that you are using and/or being exposed to. Each of the individual experiments that you are carrying out may have a specific set of safety hazards, and when necessary, they will be pointed out within the individual experimental descriptions within this manual or in the lab lecture.

In the laboratory, you must:

1. always wear safety glasses to protect your eyes. Make it a habit to put your safety goggles or glasses on before entering the laboratory. Of all the safety procedures, this is the one that the lab teacher will be the strictest with. ***You cannot work in the lab without glasses.***

2. never work alone. You must work in the presence of others. If you were to get hurt in the presence of other students, there would be someone there to help.

3. use the hoods as much as possible. All operations involving poisonous, hazardous or highly flammable materials **must** be carried out in a hood.

4. not use an open flame. Many organic solvents are more flammable than gasoline. There are modern ways to heat things such as with the use of heat guns, ovens and vacuum ovens.

5. use proper gloves. Be very careful with this safety precaution. In order for gloves to be effective at protecting your hands, they must be the proper gloves. For example, latex gloves do a great job at protecting your hands from aqueous solutions. However, they are absolutely horrible at protecting your hands from most solvents. The latex is not a barrier to most organic solvents. When working with highly volatile organics, it would have been better to not wear gloves. The organic solvent would have contacted the hand and evaporated over a relatively short period of time as opposed to being trapped in the glove over a prolonged period. For organics, nitrile rubber gloves are recommended.

6. dispose of chemicals properly. Do not throw solvents and reagents into the sink! Waste containers are located in the laboratory. If you are unable to find the waste container or if the

waste container is full, consult a teaching assistant or your lab instructor. Be sure to record what you put in the waste containers.
7. be organized and neat. Return things to storage areas after use. Everyone should clean up their laboratory area immediately after use. Do not leave your samples lying around the laboratory. Your group will be assigned a laboratory drawer in order to store such things. ***CLEAN you laboratory glassware immediately after use.***
8. use common sense. Plan your experiment in detail and think during your work. Never hesitate to ask for help or advice from the teaching assistants or your lab instructor.
9. never rush. Doing things in a hasty manner does not necessarily mean that they will get done more quickly. Quite often, rushing leads to errors which could result in a failed experiment, breaking valuable laboratory glassware, or something worse like spilling chemicals on yourself or others. Be calm.

Course Requirements: Bound laboratory notebook, written report from each experiment with full characterization of synthesized compounds, **and submission of the samples**. The report and the product should be submitted within two weeks after completion of the experiment. Points will be subtracted for late submissions.

Attendance Policy: Lectures and labs must be attended; lab make-ups are possible at the discretion of the lab instructor.

It is absolutely forbidden to work in the lab without supervision.

The course syllabus provides a general plan for the course, deviations may be necessary.

Grading

Participation: 50 points
This part of the grade is based upon your level of involvement in the laboratory including understanding of the synthetic work and work-up. Any volunteer work, such as preparation of cyclopentadiene for the class (see ferrocene), will certainly result in assigning additional points. Teaching assistants and your lab instructor will play a major role in this rather subjective evaluation.

Laboratory Notebooks: 50 points
This grade is solely based upon your neatness and conciseness of keeping a laboratory notebook for the course. Everyone is expected to keep a laboratory notebook. The writing inside of each laboratory notebook should not be the same. We all describe things in different ways and to different levels of detail. You have to be writing experimental observations in your laboratory notebook as the experiment is taking place. Laboratory notebooks will not be graded after each experiment since most laboratories may take some time to complete and interpret the results. Lab notebooks will be collected from time to time for evaluation.

Experimental reports: 100 points each (500 points total for 5 reports)
The report for each experiment should consist of the following sections:
1. *Title and Date*
2. *Objective:* Describe briefly in one or two sentences the objective of the experiment. Draw the relevant reactions and schemes.
3. *Reagents:* Record necessary physical and safety data.
4. *Procedure:* Record in a step-wise fashion (as you do it!) how the experiment is performed. Convert all quantities to experimentally and theoretically useful values (moles - mass - volume). Record what actually happens during the experiment: precise reaction times, amounts of reagents, temperatures, color changes, gas evolution, and any deviation from the provided procedure.
5. *Characterization:* Record pertinent data: yield, melting point, appearance of product. This section should include IR, NMR, and mass spectra (including assignment of signals), and chromatograms.
6. *Discussion:* Discuss briefly the results and conclusions from the experiment. Draw detailed schemes, if necessary, and discuss the mechanism Address yields and explore possible reasons for any deviation from the expected results. The first three sections should be completed prior to performing the experiment. Observations should be recorded during the experiment and characterization and discussion completed after all data is collected.
7. References: These should be indicated in the text by numerical superscripts and collected at the end of the report using an ACS format – see references in this lab manual.

The report should be structured as an ACS synthetic publication; *J. Org. Chem*. or *J. Am. Chem. Soc*. should be consulted for the required format. Briefly, the synthetic report should consist of:

1. *Title.*
2. *Summary* – This section should be brief.
3. *Introduction* – Describe briefly in one or two sentences the objective and importance of the experiment.
4. *Results and discussion* – It is often simpler to provide the results and discuss them in one section. Record pertinent data: yield, melting point, appearance of product. This section should include discussion of IR, NMR, and mass spectra (including assignment of signals), and chromatograms. Discuss the results and conclusions from the experiment. Draw detailed schemes, if necessary, and discuss the mechanism. Address yields and explore possible reasons for any deviation from the expected results. Observations should be recorded in the notebook during the experiment and characterization and discussion completed after all data is collected.
5. *Experimental section* – It should be very detailed, albeit not a mere copy of the provided experimental procedure. Record in a step-wise fashion (as you do it!) how the experiment was performed. Convert all quantities to experimentally and theoretically useful values (moles - mass - volume). Record what actually happened during the experiment: precise reaction times, amounts of reagents, temperatures, color changes, gas evolution, and any deviation from the provided procedure.
6. *References* – These should be indicated in the text by numerical superscripts and collected at the end of the report using an ACS format – see references in this lab manual.

Quizes: 50 points each (150 points total)
There will be three lab quizzes. They will contain experimental questions but some problems will require the knowledge of chemistry for explanation of the experimental features.

Final exam: 150 points, laboratory; 150 points, general lecture (300 points total)
The laboratory FE will contain practical questions about purification techniques, general laboratory procedures including handling of air-sensitive reagents, practical aspects of synthetic chemistry, and lab safety aspects. The lecture FE will contain general questions about synthetic chemistry used in the lab and elaborated upon in the lectures and specific problems such as line equations to be completed.

Summary of grading scheme

Participation		50
Laboratory notebook		50
Three lab quizzes, 3 x 50		150
Five lab reports, 5 x 100		500
Final lab exam		150
Final lecture exam		150
	Total	1050

≥ 945	A	≥ 924	A-	≥ 840	B	≥ 819	B-
≥ 770	C+	≥ 735	C	≥ 714	C-	≥ 630	D

< 630 F

CHARACTERIZATION AND IDENTIFICATION OF MOLECULES BY SPECTROSCOPIC METHODS AND ELEMENTAL ANALYSIS

Two identical compounds will show identical properties such as melting point (mp), boiling point (bp), molecular composition as determined by elemental analysis (often called microanalysis), and spectroscopic characteristics. The most common spectroscopic methods that help elucidate structure and/or identify molecules are infrared (IR) spectroscopy, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, carbon nuclear magnetic resonance (^{13}C NMR) spectroscopy, and mass spectrometry (MS). IR spectra result from absorption of energy that affects the vibrational modes of atoms that are bonded to form a molecule. ^{1}H NMR spectra result from absorption of energy that affects the spins of the magnetic hydrogen (^{1}H) atoms. ^{13}C NMR spectra are analogous to ^{1}H NMR spectra in that the magnetic carbon (^{13}C) atoms can be observed directly. Mass spectra result when molecules are ionized, which is usually followed by fragmentation of the molecular framework, and then separation of the different species based on their mass-to-charge ratio (the charge is usually +1).

In this section of the manual we will concentrate on the practical aspects of mass spectrometry including the determination of the molecular formula using high resolution MS (HR-MS). Understanding how the mass spectra are produced is essential to the successful application of mass spectrometry to organic structural analysis. The practical aspects will also guide the discussion of the NMR techniques. Although many excellent textbooks and practical manuals on application of spectral methods in organic chemistry are available, this treatise is different in that it helps introduce the student directly to "serious" graduate work.

Mass spectrometry[1-4]

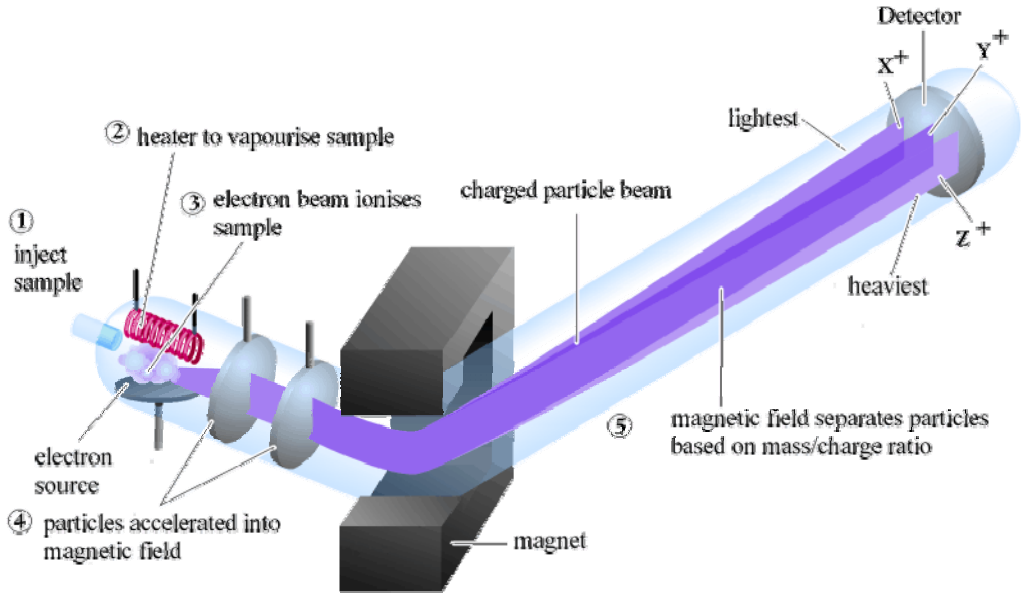


Figure 1. A schematic representation of a single-focusing mass spectrometer with an electron-impact (EI) ionization source.

Electron-impact ionization. The physics behind mass spectrometry is that a charged particle passing through a magnetic field is deflected along a circular path on a radius that is proportional to the mass-to-charge ratio, (m/z). A simple mass spectrometer is illustrated in Figure 1. This particular instrument is composed of an electron-impact (EI) ionization source, an ion accelerator, a mass analyzer, an ion detector, and a recorder. In the ionization source, the compound is introduced as a solid, liquid, or gas, with heating provided to vaporize less volatile compounds (#1,2 in Figure 1). Under a reduced pressure (10^{-4} – 10^{-6} torr), the resulting gaseous molecules then are bombarded by an electron beam from a hot filament to generate ions (#3 in Figure 1). The typical energy used is 70 eV. In a simplistic way, the chemistry involved can be described in Figure 2. Briefly, a collision of a molecule $M^{\bullet\bullet}$ with an electron causes ejection of an additional electron from the molecule and generation of a radical cation $M^{+\bullet}$. This radical cation is called molecular ion. The molecular ion can undergo fragmentation to generate an even-electron (stable) cation m_1^+ and a radical $m_1^{\bullet}$.

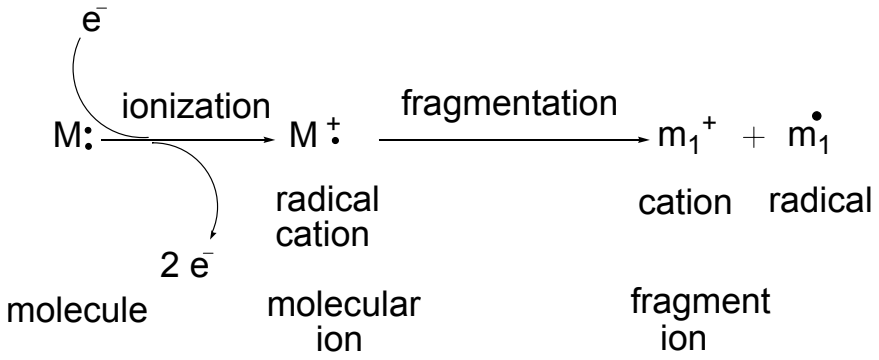


Figure 2. Ionization and fragmentation processes in the EI ionization source of a mass spectrometer.

Alternatively, the fragmentation process may generate another radical cation and a stable neutral molecule (not shown). The positive ions thus formed are directed to the analyzer tube and accelerated by attraction with negatively charged electrodes (#4 in Figure 1). The accelerated ions are not discharged at the negative plates but are "tricked" to pass through into the magnetic analyzer because the electrodes contain openings (slits). The magnetic sector analyzer is a curved tube within a magnetic field, in which the ions travel to the detector. Changing the strength of the magnetic field affects the m/z value for ion reaching the detector. All ions with m/z values that do not correspond the strength of the given magnetic field will not reach the detector and will be discharged on the surface of the analyzer tube. The detector measures the relative number of ions with a particular m/z ratio reaching it. By means of electronic amplification and recording, the detector signal is used to generate the spectrum.

An electron-impact induced mass spectrum (EI-MS) of *n*-decane is shown in Figure 3 for illustration. The molecular ion peak can be seen at m/z 142, which corresponds to the molecular weight of the molecule. Note that the mass of an electron is 1/1840 of the mass of the hydrogen atom, so that the mass difference between the molecule $M^{\bullet\bullet}$ and the molecular ion $M^{+\bullet}$ is miniscule. Often the molecular ion $M^{+\bullet}$ is abbreviated as M^+, which is acceptable as

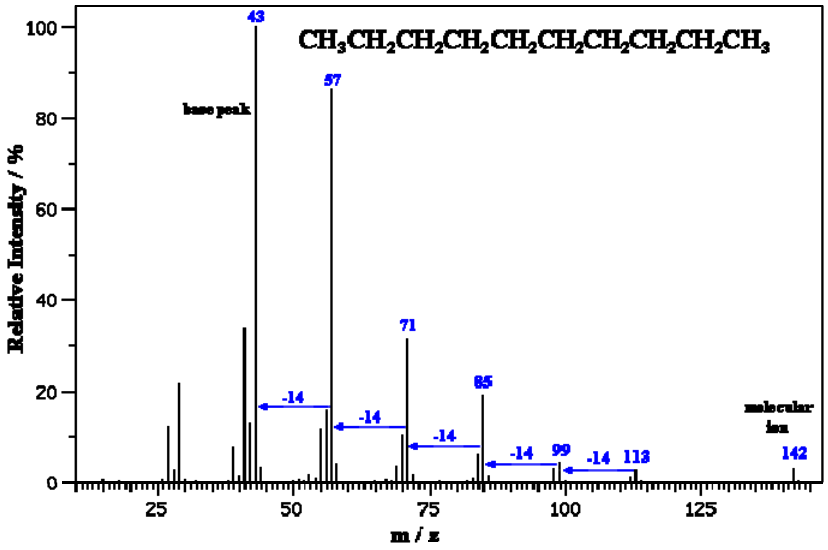


Figure 3. EI mass spectrum of *n*-decane. Note the molecular ion peak ($M^{+\bullet}$ or M^{+}) at m/z 142 and the base peak (the most intense peak) at m/z 43

long as the chemist understands that the shorter abbreviation is used for convenience exclusively. The spectra are plotted as a function of a relative intensity of peaks (abundance of ions) and the m/z values. The highest m/z value may correspond to the molecular ion peak, as in the case for *n*-decane, but the molecular ion peak is not seen if the molecular ion is unstable. The low intensity of the molecular ion peak for *n*-decane shows that a large fraction of molecular ions undergoes fragmentation and only a small fraction of the molecular ions reaches the detector. Many fragment ions are observed in the spectrum of Figure 3. The peak of the highest intensity in the spectrum is arbitrarily assigned intensity 100% and is called base peak. The terminology is summarized as follows.

Molecular ion ($\mathbf{M^{+\bullet}}$ or $\mathbf{M^{+}}$) – The ion generated by the loss of an electron from the molecule ($M^{\bullet\bullet}$).
Radical cation – Positively charged species with an odd number of electrons.
Fragment ions – Lighter cations formed by the decomposition of the molecular ion. These often correspond to stable carbocations.
Base peak – The most intense peak in the mass spectrum, assigned 100% intensity.

The spectrum in which the molecular ion peak is also the base peak indicates high stability of the molecular ion. Nevertheless, fragmentation pattern is often used for structure determination. For example, the spectrum of Figure 3 is typical for a linear hydrocarbon. The first fragment ion peak at m/z 113 indicates the loss of the ethyl radical ($M^{+\bullet}$ - 29) from the molecular ion. Other fragment ions are formed by loss of propyl, butyl, pentyl, hexyl, and heptyl radicals from the molecular ion, which corresponds to the appearance of the respective fragment ion peaks at m/z 99, 85, 71, 57, and 43. Note the mass differences of 14, corresponding to a methylene group (CH_2), between the adjacent peaks. Molecular rearrangements that are typical for carbonium ions (carbocations) are also favored in mass

spectrometry, resulting in generation of stable cations. For example, the abundant fragment ion with m/z 57 almost certainly corresponds to *tert*-butyl cation and the base peak at m/z 43 is for isopropyl cation.

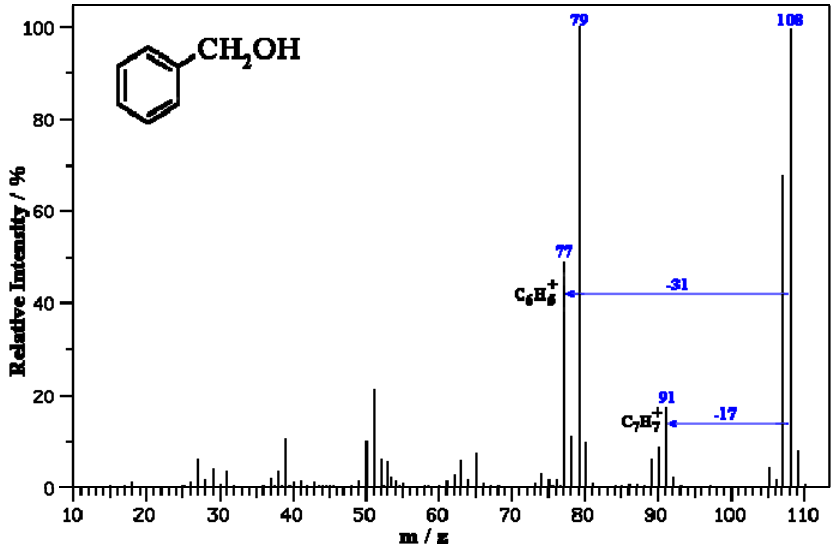


Figure 4. EI mass spectrum of benzyl alcohol

The mass spectrum of benzyl alcohol shows high stability of the molecular ion. The facile loss of a hydrogen atom from the benzylic position of the molecular ion generates a stable benzylic cation or aromatic hydroxyl-substituted cycloheptatrienyl cation (HO-$C_7H_6^+$, m/z 107). The peak at m/z 91 may correspond to the parent aromatic cycloheptatrienyl cation, $C_7H_7^+$. The loss of 17 mass units (-OH) from the molecular ion to give fragment ion, m/z 91, is typical for alcohols. The phenyl cation ($C_6H_5^+$, m/z 77) is generated by elimination of CH_2OH (31 mass units) from the molecular ion. The base peak at m/z 79 apparently corresponds to a stable albeit non-aromatic cyclohexadienyl cation which is a product of a series of rearrangements.

The spectra of Figures 3 and 4 have been obtained on a low-resolution instrument. Make no mistake, however. Low resolution means that the m/z values are accurate to the full numbers. More specifically, this means that the molecular ion peak at m/z 108 for benzyl alcohol agrees with the calculated molecular weight of benzyl alcohol using full numbers for the atomic numbers of the major isotopes present in the molecule, namely, ^{12}C, ^{16}O, and ^{1}H. The experimental value m/z 108 means just that – 108 and not 107 or 109, as it would be implied by the term "low resolution". The low resolution mass spectra of different compounds with the same nominal molecular weight are often similar and cannot be used to differentiate between them. Examples are the mass spectra of cyclohexane and 3,4-dihydro-2*H*-pyran with the molecular ion peak at m/z 84 and fragment ion peaks at m/z 69, 55, and 41 in both cases. However, many isotopes leave an unambiguous fingerprint in the mass spectra (Table 1).

Cyclohexane 

3,4-Dihydro-2H-pyran 

Table 1. Selected elements and their isotopes

Element	Atomic weight	Isotope	Abundance	Mass
Hydrogen	1.00797	^{1}H	99.985	1.00782
		^{2}H	0.015	2.01410
Carbon	12.01115	^{12}C	98.90	12.00000
		^{13}C	1.10	13.00336
Nitrogen	14.0067	^{14}N	99.634	14.00307
		^{15}N	0.366	15.00010
Oxygen	15.9994	^{16}O	99.762	15.99491
		^{17}O	0.038	16.99913
		^{18}O	0.200	17.99916
Fluorine	18.9984	^{19}F	100.00	18.9984
Sulfur	32.064	^{32}S	95.02	31.97207
		^{33}S	0.75	32.97146
		^{34}S	4.21	33.96786
Chlorine	35.453	^{35}Cl	75.77	34.96885
		^{37}Cl	24.23	36.96590
Bromine	79.909	^{79}Br	50.69	78.91839
		^{81}Br	49.31	80.91642
Iodine	126.904	^{127}I	100.00	126.90447

Let's summarize the resolution of individual ions by low-resolution mass spectrometry including information presented in Table 1.

- Low-resolution mass spectrometer is capable of separating and detecting individual ions even those that only differ by a single atomic mass unit.
- As a result, molecules containing different isotopes can be distinguished.
- This is most apparent when atoms such as bromine or chlorine are present. Natural bromine contains two isotopes, ^{79}Br and ^{81}Br, with the relative intensity of 1:1. Chlorine contains two isotopes, ^{35}Cl and ^{37}Cl, with the relative intensity of 3:1. Accordingly, the mass spectrum of a molecule containing a single chlorine atom will show two molecular ion peaks for $^{35}Cl\text{-}M^{+\bullet}$ and $^{37}Cl\text{-}M^{+\bullet}$ in the ratio of 3:1. The mass spectrum of a molecule containing a single bromine atom will show two molecular ion peaks for $^{79}Br\text{-}M^{+\bullet}$ and $^{81}Br\text{-}M^{+\bullet}$ in the approximate ratio of 1:1.

- Once more: The intensity ratios in the isotope patterns are due to the natural abundance of the isotopes.
- The low-intensity "M+1" peaks are seen due the presence of ^{13}C and ^{2}H atoms in the molecule.

The spectrum of 2-chloropropane is shown in Figure 5 for illustration. Note the isotope pattern at m/z 78 and m/z 80 for ^{35}Cl-$M^{+\bullet}$ and ^{37}Cl-$M^{+\bullet}$ in a 3:1 ratio. In order to simplify the presentation, the former molecular ion with a single ^{35}Cl isotope is abbreviated as M^+ and the latter molecular ion containing a ^{37}Cl isotope is abbreviated as M^++2. The loss of 15 mass units (methyl radical) from the molecular ions generates two ions of m/z 63 and m/z 65 in a ratio of 3:1, which means that these fragment ions still contain chlorine. By contrast, the base peak in the spectrum at m/z 43 is due to the loss of ^{35}Cl and ^{37}Cl from the respective molecular ions of m/z 78 and m/z 80. Note that the base peak at m/z 43 does not have a companion peak at m/z 45 indicating the absence of chlorine. Obviously the base peak corresponds to the stable isopropyl cation. The only isotopes in the fragment ion of m/z 43 are ^{13}C and ^{2}H atoms, as evidenced by the presence of the low intensity peak at m/z 44. Since the natural abundances of these isotopes are extremely low (see Table1), the probability of the presence of the two isotopes simultaneously in the ion is close to zero and, therefore, a peak at m/z 45 (43 + 2) is not seen.

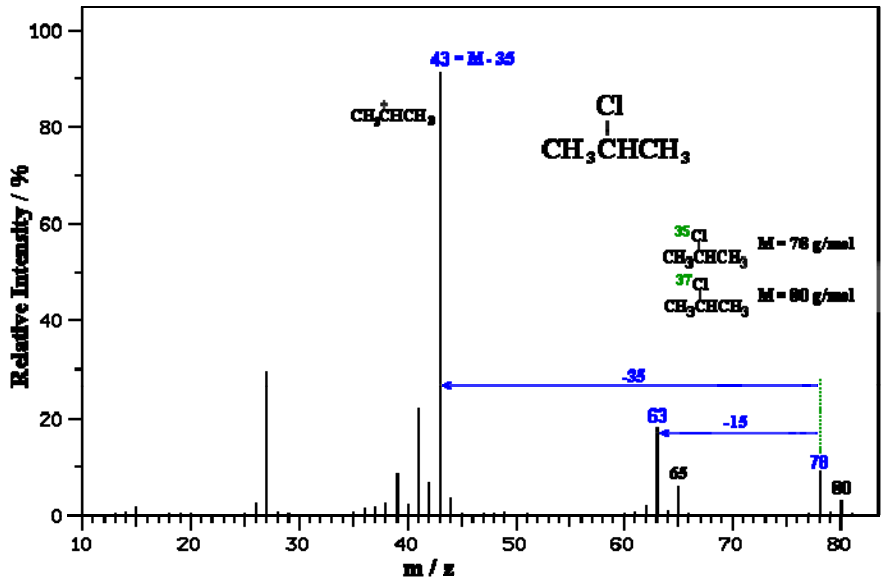


Figure 5. EI mass spectrum of 2-chloropropane.

The mass spectrum of 1-bromopropane is shown in Figure 6. Note the isotope pattern at m/z 122 and m/z 124 which corresponds to the presence of M^+ and M^++2 in a 1:1 ratio. Loss of ^{79}Br from the molecular ion of m/z 122 and ^{81}Br from the molecular ion of m/z 124 corresponds to the base peak at m/z = 43. Almost certainly the most abundant ion with m/z 43 is the more stable isopropyl cation resulting from rearrangement of the less stable *n*-propyl

cation. Rearrangements to more stable cations are common features in the chemistry taking place in a mass spectrometer. The error in Figure 6 is being discussed in this text but has not been corrected in the Figure to alert the reader about the importance of the rearrangements. The formation of stable isopropyl cation is a driving force of the fragmentation pattern of Figure 6. Note a minor fragmentation pattern that involves the loss of methyl radical (M^+ -15 = m/z 107 and 109) and ethyl radical (M^+ - 29 = m/z 93 and 95). The 1:1 intensity ratio for the presence of bromine in the corresponding fragment ions can be seen.

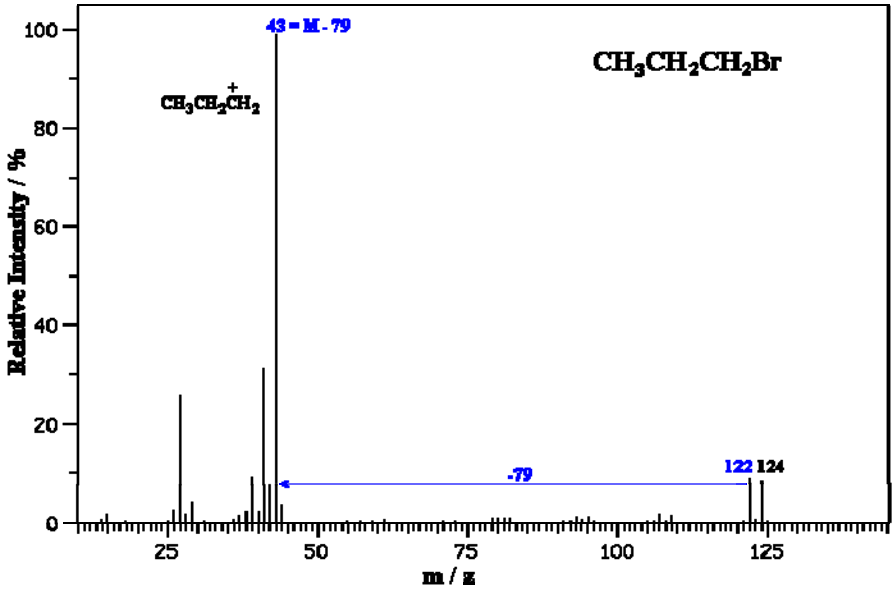


Figure 6. EI mass spectrum of 1-bromopropane.

Sulfur is another element the presence of which in the molecule can be clearly recognized by a brief inspection of the mass spectrum. As can be seen from Table 1, the major isotope ^{32}S (abundance 95%) is accompanied by a substantial amount (abundance 4.2%) of the isotope ^{34}S. Although the latter number may appear to be small, keep in mind that the mass spectra of other common molecules, excepting the presence of chlorine and bromine, do not exhibit any substantial peak at the m/z value of M^++2. The spectrum of n-butyl isopropyl sulfide is illustrative (Figure 7). The molecular ion peak at m/z 118 (^1H, ^{12}C, ^{32}S) is accompanied by an isotope peak at m/z 119 (^2H, ^{13}C, ^{33}S), and another isotope peak at m/z 120 which can be clearly correlated with the presence of ^{34}S. Note that several fragment ions also contain sulfur. As in the spectra of Figures 5 and 6, the base peak at m/z 43 is for isopropyl cation.

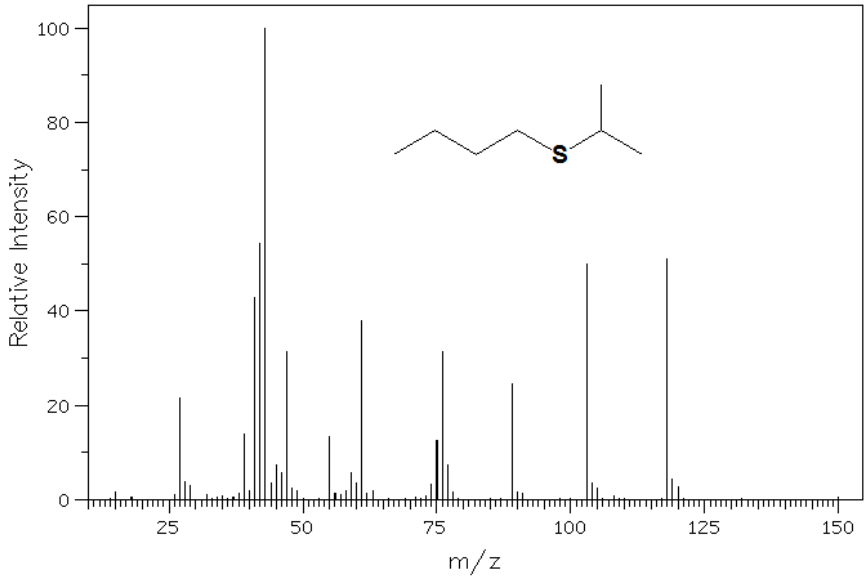


Figure 7. EI mass spectrum of *n*-butyl isopropyl sulfide

The picture becomes much more complicated when one considers the relative abundances of ions containing several polyisotopic elements. For example, the presence of two bromine atoms in an ion gives rise to three peaks at M^+ ($2^{79}Br$), M^++2 (^{79}Br and ^{81}Br), and M^++4 ($2^{81}Br$), the relative intensities being 1:2:1. In a similar way, for three bromines the peaks arise at M^+, M^++2, M^++4, and M^++6, with relative intensities 1:3:3:1. These figures ignore any insignificant contributions from ^{13}C and ^{2}H. For each element in a given ion, the relative contributions to M^++1, M^++2 peaks, etc., can be calculated from the binominal expansion of $(a + b)^n$, where a and b are the relative abundances of the isotopes and n is the number of these atoms present in the ion. Thus for three chlorine atoms in an ion, expansion gives $a^3 + 3a^2b + 3ab^2 + b^3$. Four peaks arise. The first contains three ^{35}Cl isotopes and each successive peak has ^{35}Cl replaced by ^{37}Cl until the last peak contains three ^{37}Cl isotopes. The m/z values are separated by two mass units, at M^+, M^++2, M^++4, and M^++6. Since the relative abundances of ^{35}Cl and ^{37}Cl are 3:1 ($a = 3$, $b = 1$), the intensities of the four peaks are $a^3 = 27$, $3a^2b = 27$, $3ab^2 = 9$, $b^3 = 1$ (27:27:9:1). Again, this analysis ignores minor contributions from other isotopes.

Other types of ionization. Until fairly recently, volatile compounds were ionized primarily in the electron impact ionization (EI) source, which is still the most common ion source used in gas chromatography/mass spectrometry (GC/MS) work. As the number of larger and less volatile molecules requiring analysis by mass spectrometry has grown, additional ionization techniques have been developed. Some of these new ionization methods are now used so routinely that a brief description of them is warranted. The ionization techniques described below are often referred to as *soft ionization,* because they impart significantly less energy to analyte molecules than do interactions with high-energy electrons, so that the resulting ions have little excess internal energy. These ions fragment less than those formed by EI-MS. As a result, the ionization methods described below are useful for determination of the molecular weight of molecules that do not produce a detectable $M^{+\bullet}$ by

EI-MS. Special techniques have been developed for obtaining mass spectra of non-volatile compounds including polymers.

Chemical ionization (CI). In electron-impact ionization mass spectrometry (EI-MS) discussed above the molecules are ionized through interaction with high-energy electrons (typically 70 eV). The ionization in chemical ionization mass spectrometry (CI-MS) depends on collisions of ions and molecules. In positive ion CI-MS the sample is ionized by reaction with ions generated within a large excess of a low molecular weight reagent gas such as methane (as CH_5^+) or ammonia (as NH_4^+). In CI-MS the partial pressure of analyte molecules ($\sim 10^{-3}$ torr) is small compared to the partial pressure of the reagent gas molecules (~1 torr). Thus, upon impact with high-energy electrons the reagent gas molecules are ionized preferentially. Analyte molecules are ionized by a secondary reaction with reagent gas ions, rather than directly by the electron impact. Most reagent gas ions are strong proton donors and their interaction with analyte molecules generate so called pseudomolecular ions that have a mass greater by one unit than that of the molecular mass of the starting compound. The CI method typically generates low-energy protonated ions MH^+ that undergo little fragmentation. The peak corresponding to MH^+ is usually the base peak in the spectrum. The chemistry involved is illustrated in Figure 8 for methane as the reagent gas.

$$CH_4 + e^- \rightarrow CH_4^{+\bullet} + 2e^-$$
$$CH_4^{+\bullet} + CH_4 \rightarrow CH_5^+ + {}^{\bullet}CH_3$$
$$M^{\bullet\bullet} + CH_5^+ \rightarrow MH^+ + CH_4$$

Figure 8. Chemical ionization of the molecule $M^{\bullet\bullet}$ in the presence of methane as the reagent.

*Electrospray ionization (ESI)***.** Many large molecules are nonvolatile or thermally unstable. Their mass spectra cannot be obtained by using the ionization techniques discussed above. Such compounds are easily separated by high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) and their solutions, after separation, can be used directly for ionization of the analyte molecules by using electrospray ionization (ESI) technique. The ESI source has allowed LC/MS and CE/MS to become routine analytical tools. Basically, ESI works by converting the HPLC or CE effluent, already containing the sample in ionic form, into an aerosol in a chamber under high voltage conditions. In practice, the liquid sample is sprayed from a nebulizing needle, which creates the aerosol at the opening of a capillary leading to the mass spectrometer (the m/z analyzer). As the charged droplets travel toward the capillary opening, they are subjected to the counterflow of a drying gas, typically nitrogen, which causes evaporation of the solvent molecules from the droplets. Evaporation continues until electrostatic repulsions between the increasingly concentrated charges cause the droplets to break apart. Evaporation, charge concentration, and droplets disintegration continue until the analyte ions are desorbed into the vapor phase and passed into sampling capillary leading to the m/z analyzer under extremely low-pressure conditions. In most cases, the ESI technique generates protonated molecular ions MH+ which undergo little fragmentation. Chemists Fenn and Tanaka shared the 2002 Nobel Prize in chemistry for

their development if this ingenious method of obtaining mass spectra of nonvolatile compounds.

Matrix-assisted laser desorption/ionization (MALDI). Like ESI, MALDI has proven very effective for analysis of large biopolymers. Laser desortption ionization occurs when the sample is irradiated with an intense beam of photons. Ionization of the molecules and desorption of the ions are facilitated by mixing an aqueous solution of the analyte with an excess of a compound that enhances light absorption (matrix), then placing this mixture on a probe and evaporating water. The MALDI process generates protonated molecular ions MH^+ that are relatively stable and which undergo little fragmentation. The exact mechanism by which ionization occurs is not fully understood. This ionization method is often used in conjunction with the time-of-flight analyzer (TOF), which provides highly accurate m/z values. Ion separation in a TOF analyzer is based on the principle that ions which are given the same initial energy will have velocities that are proportional to their m/z values. In the ion source of a TOF instrument , ions of all m/z values are formed almost simultaneously using a very brief burst of energy. Note that the laser burst of energy using MALDI is especially practical for the ion generation in the TOF instrument.

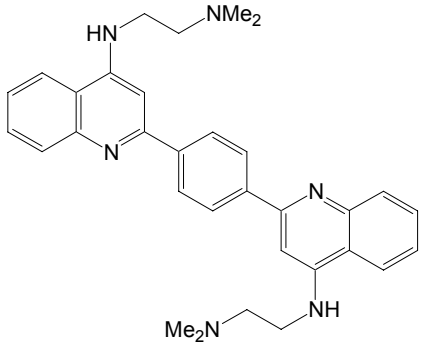
Fast atom bombardment (FAB). A needle probe is immersed in a solution containing a high molecular weight analyte and then the solvent is evaporated. A beam of energetic atoms, typically Ar*,* is sprayed onto a probe with the adsorbed analyte molecules to induce ionization of the molecules and desorption of the resultant ions. The ions are forced to travel to the m/z analyzer as described above. The spectrum contains mostly the molecular ion peak and shows little fragmentation. Although the FAB technique is experimentally simple and reliable, in recent years it was replaced by MALDI as the preferred ionization method.

High-resolution mass spectrometry (HR-MS). The single magnetic-sector mass spectrometer can unmistakably distinguish between ions differing in one mass unit. By adding an electric-field sector in tandem to the low resolution magnetic-field sector of the mass spectrometer (Figure 1), the resolution of ions is improved enormously. Such instrument is called a double focusing mass spectrometer. Its mass resolution can be accurate up to six decimal places. A question for you without the answer provided: How do you think were the isotope masses of individual isotopes of Table 1 measured? It was mentioned above that cyclohexane and 2,3-dihydro-2*H*-pyrane, both with a molecular mass of 84, cannot be distinguished by using low-resolution mass spectrometry. However, the two compounds can easily be identified by using HR-MS. Give your brain some exercise and show how. Make sure you use masses for the major isotopes that are given in the last column of Table 1 (^{1}H, ^{12}C, ^{16}O) – remember – the low resolution mass spectrometer can distinguish between masses differing in one unit, and we are using now highly accurate HR-MS. Do not even think about considering the atomic weights for elements which are shown in column 1. These are atomic weights for natural combinations of isotopes. Keep in mind that any mass spectrometer will resolve these natural mixtures into ions containing individual isotopes by providing the corresponding m/z values. The exercise above will result in the calculated values for m/z of each compound. You will be surprised to see that they differ substantially from each other. In the real world chemistry these calculations would have to be followed by taking the mass spectra of the individual compounds and them comparing the obtained m/z values with the calculated values. A difference of less than 5 parts-per-million (ppm) between the calculated and experimental values is usually acceptable for the molecular mass identification.

The following examples illustrate the determination of the molecular composition of the molecular ion $M^{+\bullet}$ or $M^{+\bullet}+H$ by using HR-MS. There is no structural information in these data, only the molecular formula. The molecular structure can be then determined by using other types of spectroscopy, such as IR, ^{1}H NMR, and ^{13}C NMR, in addition to chemical methods.

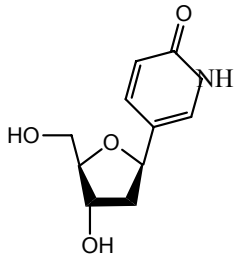
Example 1. This bis-quinoline has been synthesized and an EI mass spectrum (70 eV) obtained by a graduate student at Georgia State. The observed mass at the HR mass spectrum at m/z 504.29931 has been suggested by the computer to correlate with two molecular formulas:

$C_{31}H_{43}N_7$, calculated m/z 504.28757, (504.28757 – 504.29931)/504 = -0.0000233 or -23.3 ppm difference between the calculated value and the experimental value;
$C_{32}H_{36}N_6$, calculated m/z 504.30015, (504.30015 – 504.29931 = 0.0000017 or -1.7 ppm difference between the calculated value and the experimental value.



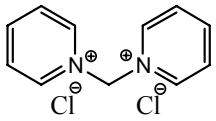
The second molecular formula is that for the given structure which, together with the low error of the mass measurement (1.7 ppm), suggest that the desired compound has been synthesized. Note that additional work has to be conducted to determine the structure.

Example 2.[5] This pyridine nucleoside has been synthesized and the peak of the highest value m/z 212.0920 in the spectrum has been analyzed in the high-resolution mode, in order to support the molecular formula $C_{10}H_{13}NO_4$. The spectrum has been obtained with the FAB ionization. The obtained molecular mass of m/z 212.0920 agrees well with the calculated value of m/z 212.0923 for $M^{+}+H$ (1.4 ppm difference).



Example 3.[6] The dipyridinium compound shown below precipitates from a solution of pyridine in dichloromethane left for several days. This reaction is a warning that chlorinated

solvents and amines are not always compatible, for example in the use as eluents in chromatography. High-resolution mass spectrometry in the ESI mode has been used to support the structure.



Note that ESI has been used because this is a highly polar, non-volatile salt. The experimental value m/z 86.04937 agrees well with the dicationic (z = 2) mass (m = 172.09874), calculated m/z 86.04948 for M^{2+} ($C_{11}H_{12}N_2$). The presence of chloride counter-anions cannot be observed by a positive ion mode mass spectrometry. This example shows that mass spectrometry sometimes cannot be used to determine the molecular composition. In addition, many compounds crystallize with one or several molecules of water in the crystal unit. The composition of such crystalline material cannot be determined by mass spectrometry. The exact composition of a solid material is important in drug discovery studies, for example, where the biological activity is analyzed as a function of the concentration of the active compound. The classical elemental analysis or microanalysis is still the only method for the quantitative determination of the atomic composition.

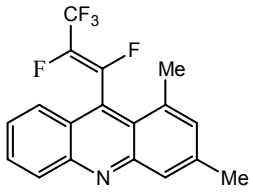
Elemental analysis (microanalysis or combustion analysis)

In addition to the limitations of HR-MS for the determination of the elemental composition of a bulk substance, as discussed above, consider a chemically pure compound mixed with an inert material such as sand. In spite of the fact that the sample is not homogeneous, mass spectrometry would give a perfect composition of the volatile component. Does it mean that the sample is composed 100% of the identified compound? Of course, not. These examples show the importance of identifying not only the molecular composition of the major component but also its content in the mixture. Although few organic compounds can be obtained with purity approaching the absolute value 100%, the purity exceeding 97% is quite common. The analysis can be conducted by gas chromatography (GC) or high-performance liquid chromatography (HPLC) methods, which resolve the mixture into individual components. The presence of one peak in the chromatogram strongly suggests high purity of the sample. Please note the use of the word "suggests" because there is no guarantee that the used chromatographic method results in a complete separation of all components. Another approach involves the use of ^{1}H NMR and ^{13}C NMR spectroscopy (see below). Briefly, the high purity of the sample is consistent with the presence of signals that can be correlated with the given structure. Any additional signals would indicate the presence of impurities. In this respect, the ^{13}C NMR spectroscopy is especially "brutal" in identifying impure samples. Elemental analysis is another method that quickly provides elemental composition for pure compounds. Conversely, an incorrect elemental analysis for a sample of the expected composition indicates the presence of impurities.

An elemental analyzer is composed of a combustion chamber coupled with a gas chromatograph capable of resolving and quantifying CO_2, H_2O, and N_2. A small sample (1 – 3 mg) of the analyzed material is placed in a tiny container made of an aluminum foil (L ~8 mm, W ~3 mm, H ~2 mm) and accurately weighed on an electronic balance. The sample is

then introduced into the combustion chamber of the elemental analyzer and burned in oxygen. The products are passed over special reagents and catalysts to ensure the final presence of CO_2, H_2O, and N_2 only. These gaseous products are then separated on a gas chromatograph with helium as a carrier gas and quantified using a thermal conductivity detector. The final results are expressed as percentages of C, H, and N in the original sample. Note that oxygen in the sample cannot be analyzed. In the absence of other elements the oxygen content is calculated by subtracting the percentage sum of C, H, and N from 100%. Special techniques are available for quantification of other elements. For example, Cl, Br, and I in a sample are reduced to the corresponding anions and then quantified gravimetrically (by weight) as insoluble silver salts. The following examples illustrate the use of elemental analysis for the determination of the empirical formula (the lowest combination of atoms) and the molecular formula (the combination of atoms in the molecule) in conjunction with HR-MS.

Example 4. A graduate student at Georgia State University has synthesized a new compound the spectral analysis of which by ^{19}F NMR, ^{1}H NMR, and ^{13}C NMR suggested the following structure:



After purification by chromatography and then crystallization, the product appeared to be pure [one spot on thin layer chromatography (TLC) plate]. Therefore, it has been decided to determine its empirical formula. The elemental analysis gave the following results: C, 63.94; H, 3.48; N, 4.07. Note that the symbol % is normally omitted. The fluorine content can be calculated as 100 – 63.94 – 3.48 – 4.07 = 28.51. In order to calculate the molar percentage of atoms in the molecule, the weight percentage of each individual element (by weight, as referred to 100 g or 100%) is divided by the respective atomic weight:

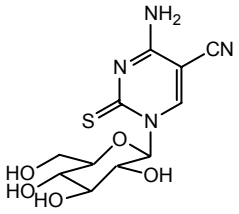
C: 63.94/12.01 = 5.324
H: 3.48/1.008 = 3.452
N: 4.07/14.007 = 0.291
F: 28.51/19.00 = 1.501

Accordingly, the number of individual atoms in the empirical formula can be calculated from the expression $C_{5.324}H_{3.452}F_{1.501}N_{0.291}$. Dividing the fractional numbers by the smallest number (0.291) leads to the empirical formula with one nitrogen atom, $C_{18.29}H_{11.86}F_{5.16}N$. By rounding the resultant numbers the following empirical formula is obtained: $C_{18}H_{12}F_5N$. This is exactly the molecular formula that is derived from the suggested structure. In conclusion, the elemental analysis results strongly support the structural information derived from spectroscopic methods.

Similar calculations should be used to obtain empirical formulas from elemental analysis results of other compounds. Since the molecular formula is the empirical formula or its multiple (M, 2M, 3M, etc.) the use of mass spectrometry in conjunction with elemental analysis can give the molecular formula, provided the molecular ion is identified. The example shown above has been taken from a real chemistry work. The final rounding of a

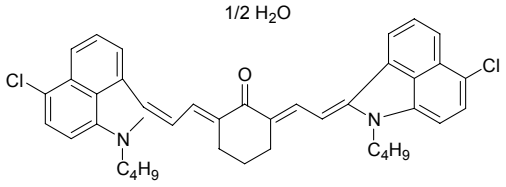
number of atoms, 18.29 to 18, 11.86 to 12, and 5.16 to 5, is a no-brain procedure. What about less accurate combustion results that would give ambiguous numbers such as 18.50 or 11.50? Such numbers would indicate an impure sample, and a chemist would have to go back to the bench and purify the compounds. Normally, highly rated chemistry journals including Journal of Organic Chemistry (J. Org. Chem.) and Journal of the American Chemical Society (J. Am. Chem. Soc.) accept for publication elemental analysis results the experimental and calculated values of which differ by less than 0.4% (the original values, referred to 100g or 100%) for C, H, and N. In order to comply with this requirement the following reporting format is used: Calculated for $C_{18}H_{12}F_5N$: C, 64.09; H, 3.56; N, 4.15. Found: C, 63.94; H, 3.48; N, 4.07. It can be seen that the differences in the discussed case are much smaller than 0.4% for all elements. Practice and calculate the theoretical values shown above. A few final words of caution about choosing atomic weights for the calculation: These are atomic weights that are average numbers for the presence of all isotopes (Table 1, column 1). Do not even think about taking atomic masses for individual isotopes. More specifically, the mass of carbon atom is 12.01 for elemental analysis (the average value for the presence of all carbon isotopes) and 12.000000 (the reference) for all mass spectrometry problems.

Example 5. Another graduate student at Georgia State University designed a synthetic scheme for the preparation of the nucleoside shown below. The synthesized compound appeared to be pure by TLC analysis (one spot using several eluents), and ^{1}H NMR and ^{13}C NMR spectra were fully consistent with the desired structure. Due to the thermal instability, no molecular ion peak was observed in the standard EI mass spectrum (other ionization methods were not available at that time). The elemental analysis results were as follows: C, 48.84; H, 5.10; N, 13.33; S, 7.73. Is the suggested structure correct? Doing the calculations you will arrive at the horrific number of 6.48 oxygen atoms (close to 6.50 – which way to round it?). The student thought the compound was not pure and subjected it to additional purification – with the same analysis results after the purification. What is going on? This is a homework for you. *Hint*: there is nothing wrong with the analysis; think about crystallization water, *e.g.* M•0.5H_2O or 2M•H_2O, which is the same – one water molecule per 2 molecules of the nucleoside in the crystal unit. Good luck with your calculations.

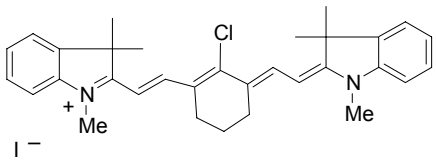


Example 6. This is another homework for you. What is the molecular formula of a compound with the following characteristics?
Low-resolution EI MS: the highest m/z value at 275.
High-resolution EI MS: m/z 275.09246
Elemental analysis: C, 69.88; H, 4.49; N, 5.03. In addition, the molecule may contain fluorine but no oxygen. This information comes from the synthetic route – no reagents with oxygen were used and the reaction was conducted under an atmosphere of nitrogen (no contact with air), one reagent contained fluorine.

Example 7.[7] (Homework). The IUPAC name of the ketone shown below is 2,6-bis[2'[1''-butyl-6''-chlorobenzo[*cd*]indol-2''-(1''*H*)ylidene]ethylidene]cyclohexanone (see the section Cyanine Dyes in this manual). In methanol it shows electronic absorption at λ_{max} 648 nm. Upon acidification, the ketone is protonated at the carbonyl oxygen atom, which results in the formation of a cyanine dye with absorption in the near-infrared region at λ_{max} 932 nm. Comment on the following analysis results of the ketone: HR-MS (FAB ionization), observed m/z 633.2456; elemental analysis results: C, 75.07; H, 6.30; N, 4.53. Comment on particular isotopes and accuracy of the measurements.



Example 8. (Homework). The structure shown below is compound **11** in the section Cyanine Dyes of this manual. See that section for the IUPAC name of **11**. Your current



assignment is to comment on the following analysis data: HR-MS (FAB ionization), m/z 483.2541; elemental analysis: C, 62.80; H, 5.98; N, 4.57. Comment on particular isotopes and accuracy of the measurements.

Nuclear magnetic resonance (NMR)[1,3]

Over the past fifty years nuclear magnetic resonance spectroscopy, commonly referred to as NMR, has become an important technique for determining the structure of organic compounds. Of all the spectroscopic methods, it is the only one for which a complete analysis and interpretation of the entire spectrum is normally expected. Although larger amounts of sample are needed than for mass spectroscopy, NMR is non-destructive, and with modern instruments good data may be obtained from samples weighing less than a milligram. *To be successful in using NMR as an analytical tool, it is necessary to understand the physical principles on which the method is based.*

Atomic nuclei that contain an odd number of protons and neutrons display a magnetic moment caused by a quantized spin of each nuclear particle. Some nuclei have integral spins I (e.g. I = 1, 2, 3 ....), some have fractional spins (e.g. I = 1/2, 3/2, 5/2 ....), and a few have no spin, I = 0 (e.g. ^{12}C, ^{16}O, ^{32}S, ...). Isotopes of particular interest and use to organic NMR chemists are ^{1}H, ^{13}C, ^{19}F and ^{31}P, all of which have I = 1/2. Our discussion of NMR will be limited to these important nuclei. Proton NMR spectroscopy (^{1}H NMR) will be emphasized.

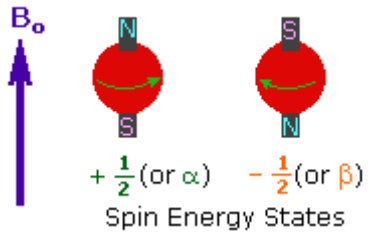


Figure 9. Magnetic field is created by a spinning charge. The resultant magnetic dipoles of nuclei (I =1/2) are aligned with the external magnetic field B_o as shown.

A spinning charge generates a magnetic field, as shown in Figure 9. The resulting spin-magnet has a magnetic moment μ that is proportional to the spin. In the presence of an external magnetic field B_0, two spin states exist, +1/2 and -1/2. The magnetic moment of the lower energy +1/2 state is positively aligned with the external field, but that of the higher

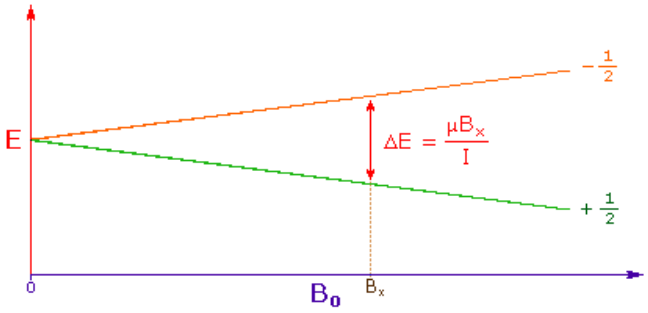


Figure 10. Splitting of energy levels for a nucleus with I = ½, such as hydrogen, in an external magnetic field.

energy -1/2 spin state is opposed to the external field. Note that the arrow representing the external field and the internal magnetic moment of a nucleus with $I = +1/2$ point to the same direction. The difference in energy between the two spin states is dependent on the external magnetic field strength, and is always very small. The following diagram in Figure 10 illustrates that the two spin states have the same energy when the external field is zero, but diverge as the field increases. At a field equal to B_x a formula for the energy difference is given (remember $I = 1/2$ and μ is the magnetic moment of the nucleus in the field).

Under the influence of an external magnetic field, a magnetic nucleus can take up different orientations with respect to that field. The number of possible orientations is given by the expression $(2I + 1)$, so that for nuclei with spin I = ½ (^{1}H, ^{13}C, ^{19}F, ^{31}P) only two orientations are allowed (Figure 9). In an applied magnetic field, magnetic nuclei like proton precess at a frequency v, which is proportional to the strength B_x of the applied field. The exact precession frequency v is given by Equation 1,

$$v = \gamma B_x / 2\pi \qquad (1)$$

where B_x = strength of the applied external magnetic field experienced by the nucleus,
γ = magnetogyric ratio, which is a characteristic constant for each magnetically active nucleus. Since $E = h\,v$ (the Einstein equation), the Equation 1 is equivalent with the equation given in Figure 9. Thus, precessional frequencies for a given nucleus increase with the increase in the strength of the external magnetic field. For example, for proton, v = 60 MHz at B_x = 1.4 tesla and v = 300 MHz at B_x = 7.1 tesla; for ^{13}C, v = 15.1 MHz at B_x = 1.4 tesla and v = 75.5 MHz at B_x = 7.1 tesla. Note that the precessional energies are about 4-fold smaller for ^{13}C than for ^{1}H at the identical strength of the external magnetic field. All these values fall in the range of radio frequencies.

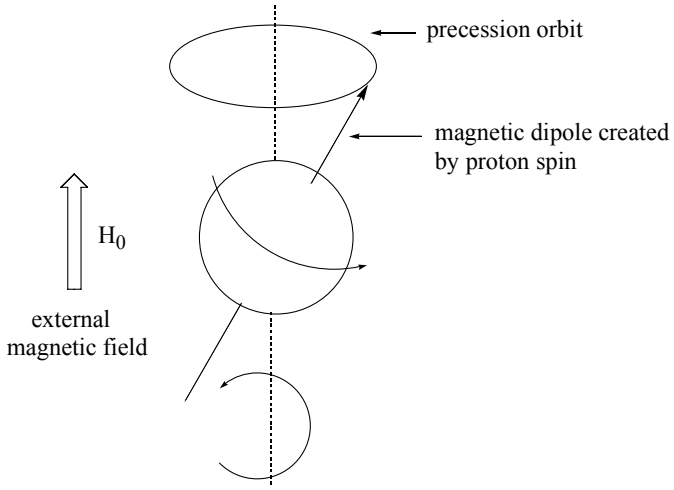


Figure 11. Precession of a magnetic nuclei (I = ½) in an external magnetic field

What happens when nuclei absorb the radio frequency energy, which takes place when the external frequency and the precessional frequency become identical? This condition is called resonance. Nuclei in the lower energy state undergo transition to the higher energy state and the populations of the two states may approach equality. When this situation arises (it is called saturation), no further absorption of energy can occur and the observed resonance

signal will fade out. In the recording of a NMR spectrum, however, the populations in the two spin states do not become equal because higher-energy nuclei are constantly returning to the lower-energy spin state. The nuclei return to the ground state by two processes called spin-lattice and spin-spin relaxation. In the spin-lattice process, the energy is transferred to the molecular framework (lattice) and is lost as translational or vibrational energy of heat. The half-life for this process is called T_1. Spin-spin relaxation occurs by transfer of the energy from one nucleus to neighboring nuclei, and the half-life of this process is called T_2.

^{1}H NMR spectroscopy. To begin with, the NMR spectrometer must be tuned to a specific nucleus, in this case the proton (see above). The schematic representation of the simplest continuous wave (CW) NMR instrument is given in Figure 12. The basic features of the instrumentation needed to record an NMR spectrum are a magnet, a radiofrequency source (the transmitter in Figure 12), and a detection system (receiver and amplifier) for absorption of the radiofrequency energy by the nucleus during resonance. A solution of the sample in a uniform 5 mm glass tube is placed between the poles of a powerful magnet, and is spun to average any magnetic field variations, including that resulting from tube imperfections. Radiofrequency radiation (e.g. 60 MHz) is broadcast into the sample from the transmitter (colored red). A receiver coil surrounds the sample tube, and emission of absorbed radiofrequency energy during resonance is monitored by dedicated electronic devices and a computer. An nmr spectrum is acquired by varying or sweeping the strength of the magnetic field (1.4 tesla in this exaple) over a small range using the sweep coils while observing the radiofrequency signal from the sample. An equally effective technique is to vary the frequency of the radiofrequency radiation while holding the external field constant (no use of the sweep coils). An increased signal will be detected if nuclei in the sample resonate with the source because energy will be transferred from the source, via the nuclei, to the detector coil.

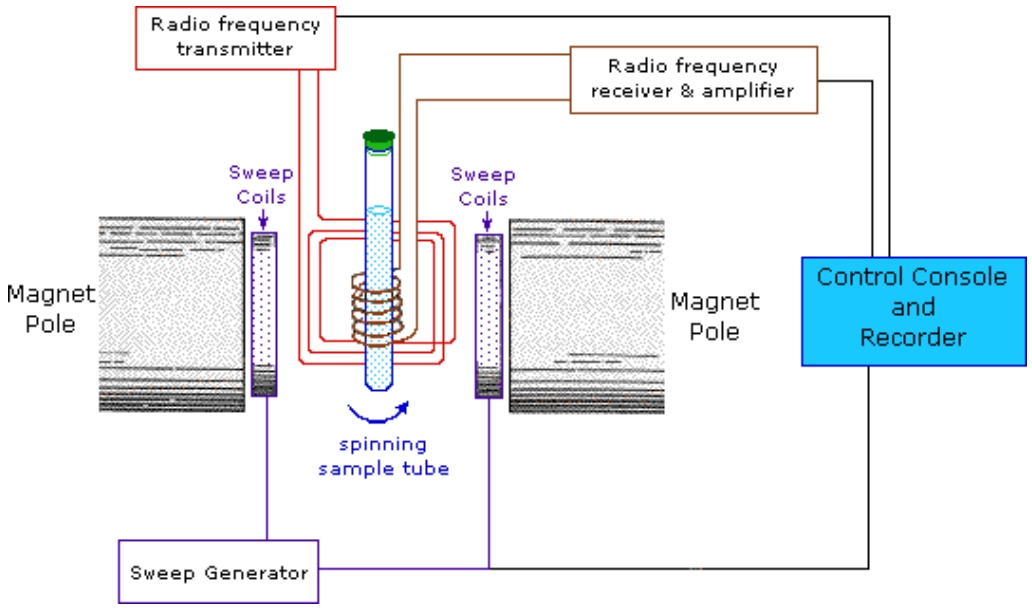


Figure 12. A diagram of a continuous wave NMR (CW-NMR) instrument. The sweep coils are used to modulate the strength of the external magnetic field.

In the highly sensitive pulsed NMR instrument the sample is irradiated at fixed magnetic field with a strong pulse of radiofrequency energy containing all the frequencies over the ^{1}H range. For example at 7.1 tesla magnetic field the frequencies are spread around 300 MHz. The protons in each environment absorb their appropriate frequencies from the pulse, and these absorptions are then analyzed by using a Fourier Transform technique. The typical pulse duration is around 10 μs, and when it is switched off, the nuclei undergo relaxation processes and lose the absorbed energies. An entire spectrum can be recorded, computerized, and transformed in a few seconds. Many spectra can be accumulated over a relatively short period of time, which greatly increases sensitivity of this method. For example, typically 400 spectra can be accumulated in approximately 13 min using a repetition every 2 sec. In practice, the pulse repetition rate depends mainly on the relaxation time T_1.

The samples. A liquid substance (about 0.7 ml) with a little TMS (Figure 13) can be poured into a sample tube and examined in an NMR spectrometer. In order to take the NMR spectrum of a solid, it is usually necessary to dissolve it in a suitable solvent. Early studies used carbon tetrachloride for this purpose, since it has no hydrogen that could introduce an interfering signal. Unfortunately, CCl_4 is a poor solvent for many polar compounds and is also toxic. Deuterium labeled compounds, such as deuterium oxide (D_2O), chloroform-*d* ($CDCl_3$), benzene-d_6 (C_6D_6), acetone-d_6 (CD_3COCD_3) and DMSO-d_6 (CD_3SOCD_3) are now widely used as NMR solvents. Since the deuterium isotope of hydrogen has a different magnetic moment and spin, it is invisible in a spectrometer tuned to protons.

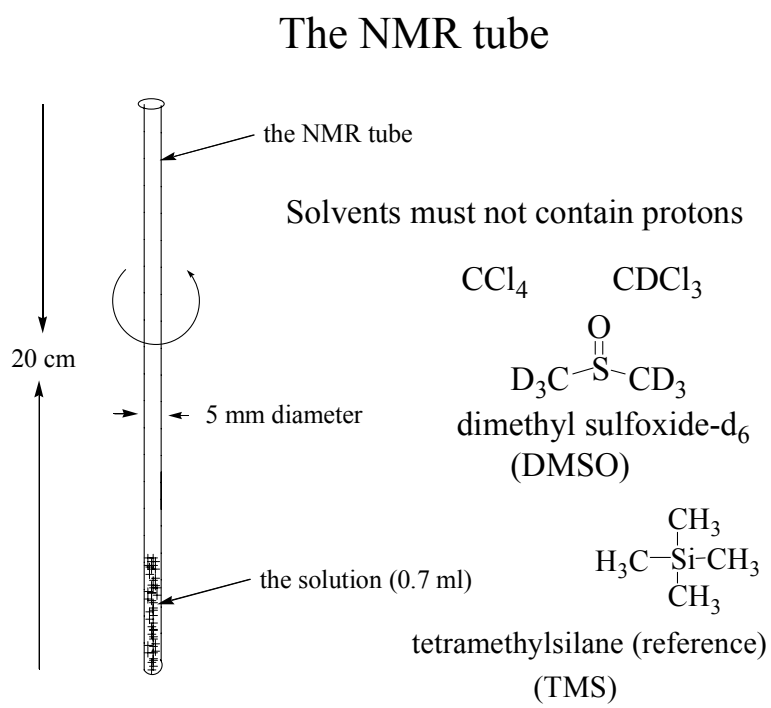


Figure 13. The NMR tube, typical solvents, and the reference (TMS).

Chemical shift. Why do the proton nuclei in different compounds behave differently in the NMR experiment? The answer to this question lies with the electrons surrounding the proton in covalent compounds and ions. Since electrons are charged particles, they move in response to the external magnetic field B_o and by doing so they generate a secondary magnetic field that (always) opposes the externally applied field. This secondary field *shields* the nucleus from the applied field, so B_o must be increased in order to achieve resonance (absorption of radiofrequency energy). As illustrated in Figure 14, B_o must be increased to compensate for the induced shielding effect. Certain structural factors in the molecule cause the opposite effect called the deshielding effect (see below "π-electron-induced deshielding").

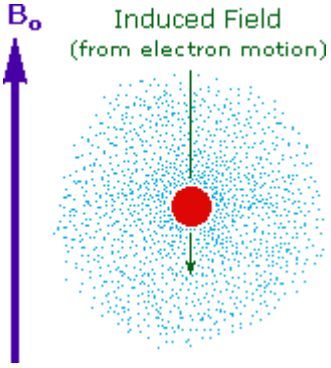


Figure 14. The shielding effect

In summary, various nuclei of the same type (e.g. protons) will show resonance at different values of the precessional frequency. The measurement of the precessional frequency in absolute frequency units is possible but impractical. More commonly, the differences in frequency are measured with respect to the frequencies of the reference nuclei. For ^{1}H and ^{13}C, the universally accepted reference is tetramethylsilane abbreviated as TMS (Figures 13 and 15). TMS is chemically inert and easily removed from the sample after the measurement. Also, it gives a single sharp NMR signal that does not interfere with the resonances normally observed for organic compounds. The frequency differences between a resonating proton of a molecule and the protons of TMS are small, typically several hertz, and they depend on the strength of the external magnetic field (Equation 1). In order to arrive at a universal measure of the resonance position that is not dependent on the magnetic field strength, the frequency difference ($\nu_{mol} - \nu_{TMS}$) is divided by spectrometer frequency ν, such as 100 MHz or 500 MHz for example, and the resultant fraction is multiplied by a million (Equation 2). This operation gives a locator number called the *chemical shift*, having units

$$\delta = (\nu_{mol} - \nu_{TMS})/\nu \times 10^6 \qquad (2)$$

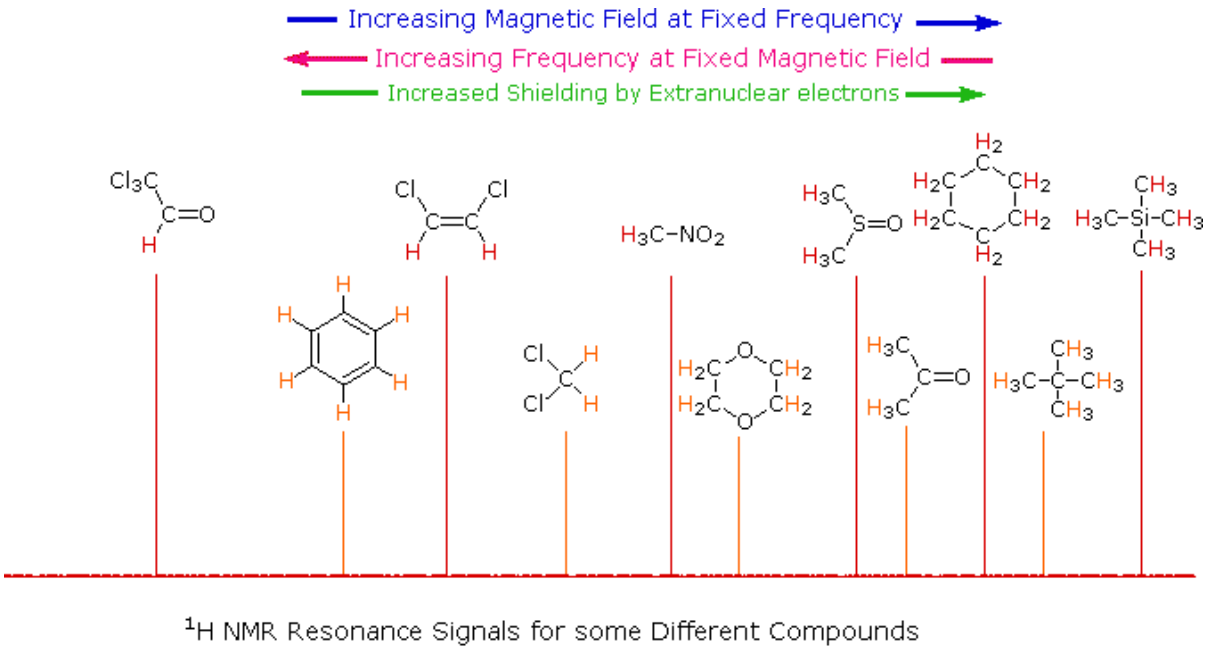


Figure 15. Relative positions for the resonance of selected protons. The resonance of the tetramethylsilane (TMS) shown on the right is universally used as a reference.

of parts-per-million (ppm), and designated by the symbol δ (delta). Note that the chemical shift would be extremely small, around 10^{-6}, without multiplying the ratio by a million. The chemical shifts of the protons of a vast majority of organic molecules, expressed by Equation 2, are in the range from 0 ppm (for TMS – signal on the right) to about 15 ppm (for carboxylic acids – values increase from the right to the left). Note that the δ values for protons of a given molecule are independent on the NMR instruments with different magnetic fields.

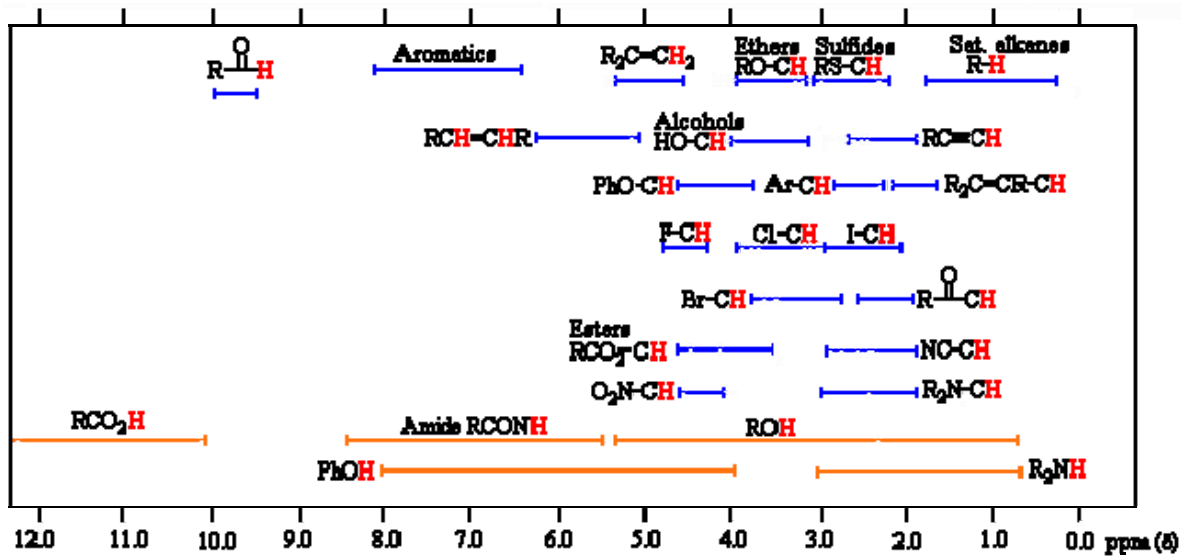


Figure 16. Proton chemical shift ranges for samples in $CDCl_3$ solution. The δ scale is relative to TMS at $\delta = 0$.

The general distribution of proton chemical shifts associated with different functional groups is summarized in Figure 16. Bear in mind that these ranges are approximate, and may not encompass all compounds of a given class. Note also that the ranges specified for OH and NH protons (colored orange) are wider than those for most CH protons. This is due to hydrogen bonding variations at different sample concentrations.

The different ranges for chemical shifts of protons arise from the following electronic phenomena within the molecular structure: (i) electronegativity of neighboring groups or atoms, (ii) hybridization, (iii) acidity and hydrogen bonding, and (iv) magnetic anisotropy. *Electronegativity* effects are the easiest to understand. If electron density is withdrawn from around the hydrogen nucleus toward a more electronegative atom, the lower electron density around this hydrogen atom will produce a smaller magnetic field (opposite to the magnetic field of the spectrometer) and, as a result, this proton will be deshielded and will resonate at a position farther downfield (farther to the left in the spectrum). For example:

CH_3-CH_3 δ 0.26 CH_3-Cl δ 3.06 CH_3-OCH_3 δ 3.24

In the *hybridization* effects the increased s-orbital contribution to the C-H bond vs. p-orbital contribution will result in less electron density in this C-H bond, hence deshielding. However, hybridization effects are often outshadowed by the magnetic anisotropy effects. *Acidity and hydrogen bonding* also cause deshielding of the protons. Finally, *magnetic anisotropy* has one of the greatest influences on the resonance. These two topics (iii and iv) are discussed in a greater detail below.

Signal strength (integration). The magnitude or intensity of NMR resonance signals displayed on a spectrum is proportional to the molar concentration of the compound. Thus, a small or dilute sample will give a weak signal, and doubling or tripling the sample concentration increases the signal strength proportionally. If we take the NMR spectrum of equal molar amounts of benzene and cyclohexane in carbon tetrachloride solution, the resonance signal from cyclohexane will be twice as intense as that from benzene because cyclohexane has twice as many hydrogen atoms per molecule. This is an important relationship when samples incorporating two or more different sets of hydrogen atoms are examined, since it allows the ratio of hydrogen atoms in each distinct set to be determined. To this end it is necessary to measure the relative strength as well as the chemical shift of the resonance signals that comprise an NMR spectrum. Two common methods of displaying the integrated intensities associated with a spectrum are illustrated in Figure 17. In the three spectra in the top row, a horizontal integrator trace (light green) rises as it crosses each signal by a distance proportional to the signal strength. Alternatively, an arbitrary number, selected by the instrument's computer to reflect the signal strength, is printed below each resonance peak, as shown in the three spectra in the lower row. From the relative intensities shown here, together with the previously noted chemical shift correlations, the reader should be able to assign the signals in these spectra to the set of protons that generates each. *Hint:* When evaluating relative signal strengths, it is useful to set the smallest integration to unity and convert the other values proportionally.

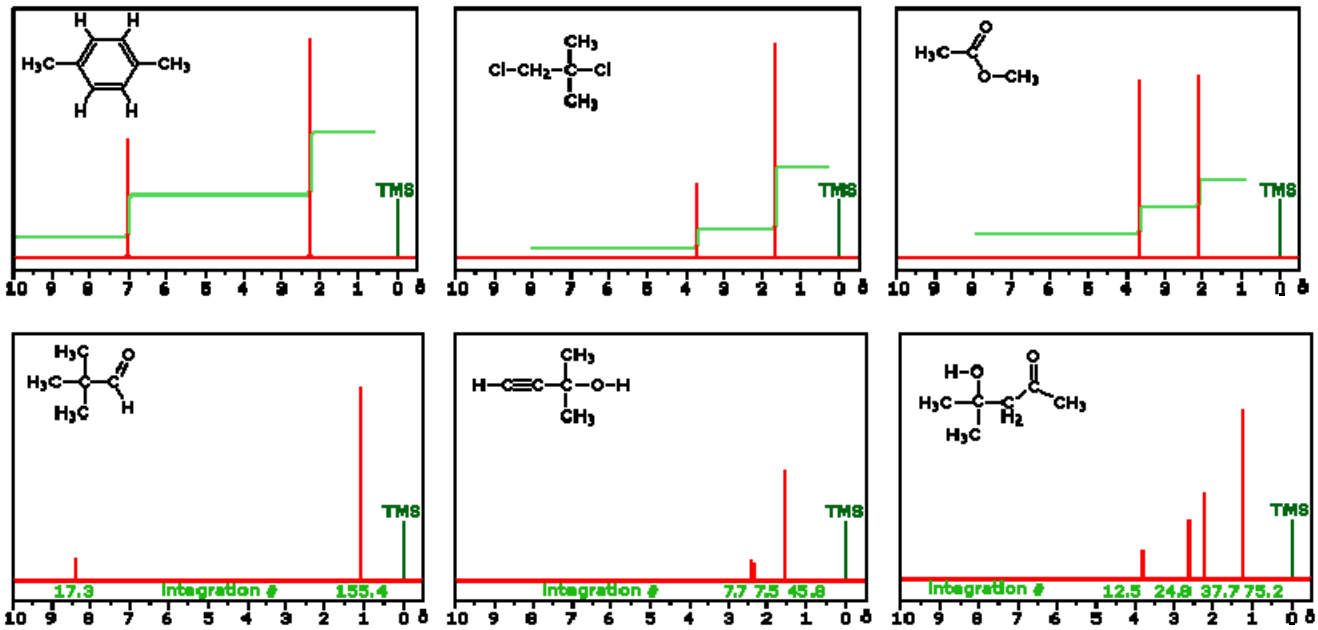


Figure 17. Integration of the NMR spectra.

Hydroxyl proton exchange and hydrogen bonding. The last two compounds in the lower row are alcohols. The OH proton signal is seen at δ 2.37 in 2-methyl-3-butyne-2-ol, and at δ 3.87 in 4-hydroxy-4-methyl-2-pentanone, illustrating the wide range over which this chemical shift may be found. A six-membered ring intramolecular hydrogen bond in the latter compound is in part responsible for the low field shift. We can take advantage of the rapid OH exchange with the deuterium of heavy water to assign hydroxyl proton resonance signals. As shown in Figure 8 below, this removes the hydroxyl proton from the molecule and its resonance signal in the NMR spectrum disappears. Experimentally, a spectrum is taken, then a drop of D_2O is added to the solution and the spectrum is taken again. The result of the H – D exchange is displayed in Figure 18.

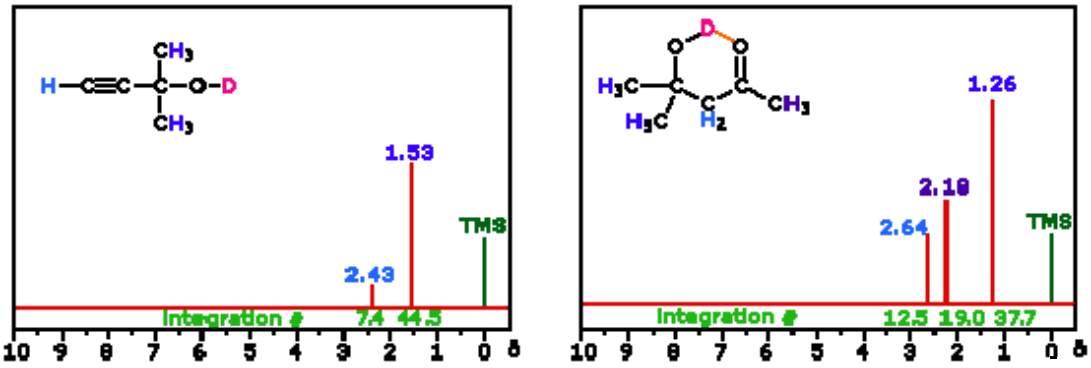


Figure 18. The effect of the H – D exchange on the NMR spectra (compare to Figure 15).

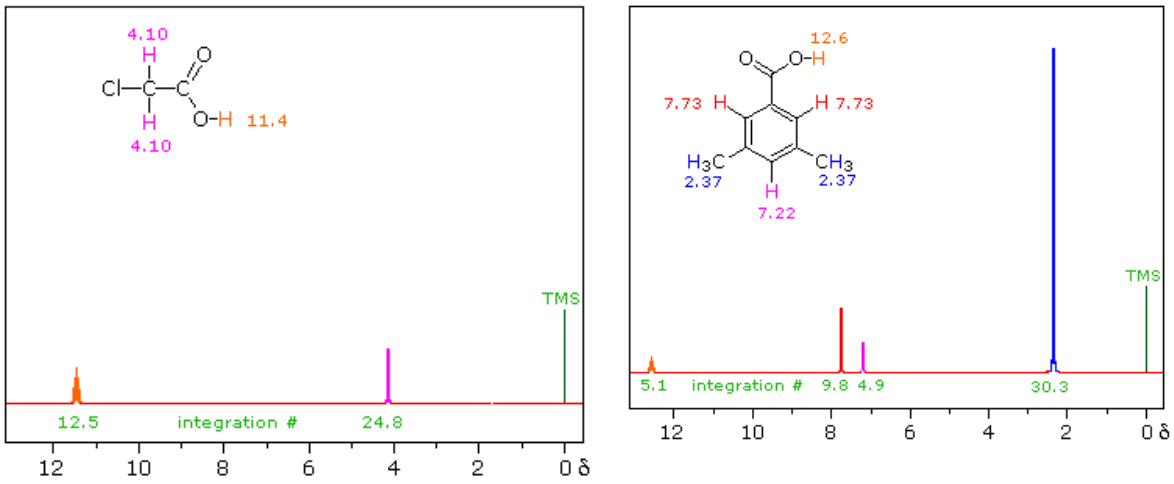


Figure 19. ^{1}H NMR spectra of chloroacetic acid and 3,5-dimethylbenzoic acid.

The hydroxyl proton can resonate over a large range of chemical shifts but hydrogen bonding results in the resonance at a lower magnetic field or higher frequency. Because of their favored hydrogen-bonded dimeric association, the hydroxyl proton of carboxylic acids displays a resonance signal significantly down-field of other functions. For a typical acid the signal appears from δ 10 to 13 and is often broader than other signals. The spectra shown below in Figure 19 for chloroacetic acid (left) and 3,5-dimethylbenzoic acid (right) are typical examples.

π-Electron-induced shielding and deshielding (magnetic anisotropy). An examination of the proton chemical shift chart (Figure 16) makes it clear that the inductive effect of substituents cannot account for all the differences in proton signals. In particular the low field resonance of hydrogen atoms bonded to double bond or aromatic ring carbons is puzzling, as is the very low field signal for an aldehyde proton. The hydrogen atom of a terminal alkyne, in contrast, appears at a relatively higher field. All these anomalous cases seem to involve hydrogen atoms bonded to pi-electron systems, and an explanation may be found in the way these π-electrons interact with the applied magnetic field. π-Electrons are more polarizable than sigma-bond electrons. Therefore, we should not be surprised to find that field-induced π-electron movement produces strong secondary fields that perturb nearby nuclei. The π-electrons associated with a benzene ring provide a striking example of this phenomenon, as shown in Figure 20. The electron cloud above and below the plane of the ring circulates in reaction to the external field so as to generate an opposing field at the center of the ring and a supporting field at the edge of the ring. This kind of spatial variation is called *anisotropy*, and it is common to non-spherical distributions of electrons. Regions in which the induced field supports or adds to the external field are *deshielded*, because a slightly weaker external field will bring about resonance for nuclei in such areas. Conversely, regions in which the induced field opposes the external field are *shielded* because an increase in the applied field is needed for resonance. Shielded regions are designated by a plus sign, and deshielded regions by a negative sign. Note that the anisotropy about the triple bond nicely accounts for the relatively high field chemical shift of ethynyl hydrogens. The shielding & deshielding regions about the carbonyl group can be explained in a similar way.

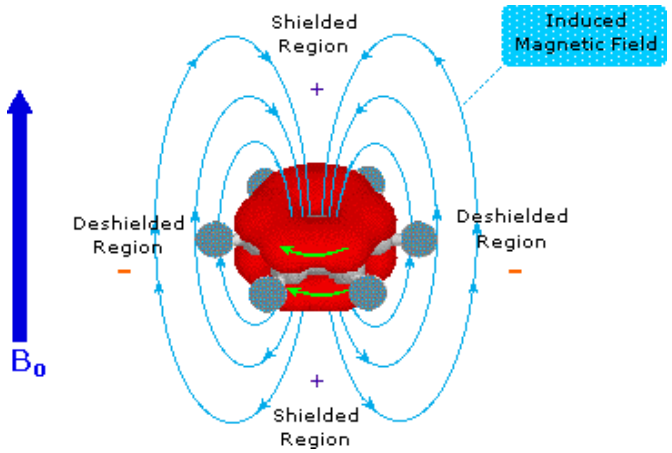


Figure 20. Magnetic anisotropy at the benzene ring.

Spin-spin interactions (coupling constants). The nmr spectrum of 1,1-dichloroethane (Figure 19, right) is more complicated than we might have expected from the previous examples. Unlike its 1,2-dichloro-isomer (Figure 21, left), which displays a single resonance signal from the four structurally equivalent hydrogen atoms, the two signals for 1,1-dichloroethane are split into close groupings of two or more resonances. This is a common feature in the spectra of compounds having different sets of hydrogen atoms bonded to adjacent carbon atoms. The signal splitting in proton spectra is usually small, ranging from fractions of a Hz to as much as 18 Hz, and is termed the coupling constant *J*. In the 1,1-dichloroethane example the coupling constant is 6.0 Hz.

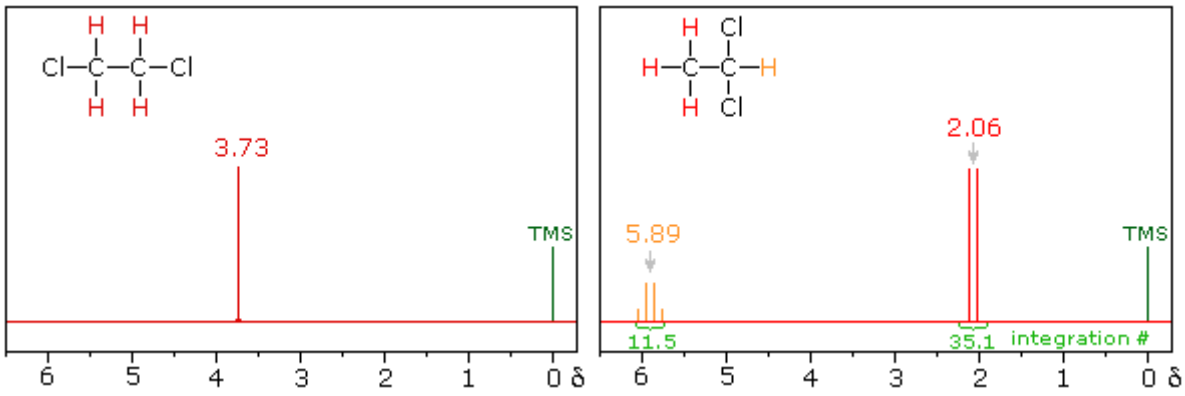


Figure 21. The spectra with and without a coupling pattern.

The splitting patterns found in various spectra are easily recognized, provided the chemical shifts of the different sets of hydrogen that generate the signals differ by two or more ppm. The patterns are symmetrically distributed on both sides of the proton chemical shift, and the central lines are always stronger than the outer lines. The most commonly observed patterns have been given descriptive names of a *doublet* (d) for two equal intensity

signals, a *triplet* (t) for three signals with an intensity ratio of 1:2:1, a *quartet* (q) for a set of four signals with intensities of 1:3:3:1, and a *quintet* for a set of five signals with intensities of 1:4:6:4:1. These patterns are displayed in the following illustration. The line separation is always constant within a given multiplet, and is called the *coupling constant (J)*. The magnitude of *J*, usually given in units of Hz, is magnetic field independent.

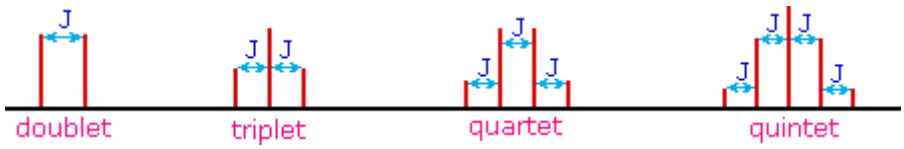


Figure 22. Typical coupling patters.

The splitting patterns shown above (Figure 22) display the ideal or *"First-Order"* arrangement of lines. This is typically observed if the spin-coupled nuclei have very different chemical shifts (i.e. $\Delta\nu$ is large compared to J). If the coupled nuclei have similar chemical shifts, the splitting patterns are distorted (second order behavior). In fact, signal splitting disappears if the chemical shifts are the same. Two examples that exhibit minor 2^{nd} order distortion are shown in Figure 23 (both are taken at a frequency of 90 MHz). The ethyl acetate spectrum in the top displays the typical quartet and triplet of a substituted ethyl group. The spectrum of 1,3-dichloropropane in the bottom demonstrates that equivalent sets of hydrogens may combine their influence on a second, symmetrically located set. Even though the chemical shift difference between the A and B protons in the 1,3-dichloroethane spectrum is fairly large (140 Hz) compared with the coupling constant (6.2 Hz), some distortion of the splitting patterns is evident. The line intensities closest to the chemical shift of the coupled partner are enhanced. Thus the B set triplet lines closest to A are increased, and the A quintet lines nearest B are likewise stronger. A smaller distortion of this kind is visible for the A and C couplings in the ethyl acetate spectrum (a roof effect). This is a valuable information showing that the two sets of protons are coupled to each other.

What causes this signal splitting, and what useful information can be obtained from it? If an atom under examination is perturbed or influenced by a nearby magnetic field caused by a nuclear spin (or set of spins), the observed nucleus responds to such influences, and its response is manifested in its resonance signal. This spin-coupling is transmitted through the connecting bonds, and it functions in both directions. Thus, when the perturbing nucleus becomes the observed nucleus, it also exhibits signal splitting with the same value of *J*. For spin-coupling to be observed, the sets of interacting nuclei must be bonded in relatively close proximity (e.g. vicinal and geminal locations, Figure 23), or be oriented in certain optimal and rigid configurations. Some spectroscopists place a number before the symbol *J* to indicate the number of bonds linking the coupled nuclei (colored orange below). Using this terminology, a vicinal coupling constant is ^{3}J and a geminal constant is ^{2}J.

Figure 23. Geminal and vicinal couplings.

Figures 24 and 25 illustrate the coupling that arises in the quartet signal of the methylene protons (CH_2) that are coupled to the adjacent methyl protons (CH_3). As can be seen from Figure 24, the equivalent CH_2 protons can 'see' eight different combinations of the spins of the methyl protons. The orientation (1) contain three protons aligned along the external magnetic field of the spectrometer, there are three orientations (2) of the same energy, three orientations (3), and one orientation (4) with all three protons aligned against the external magnetic field. All four orientations (energies) of the protons of the methyl group

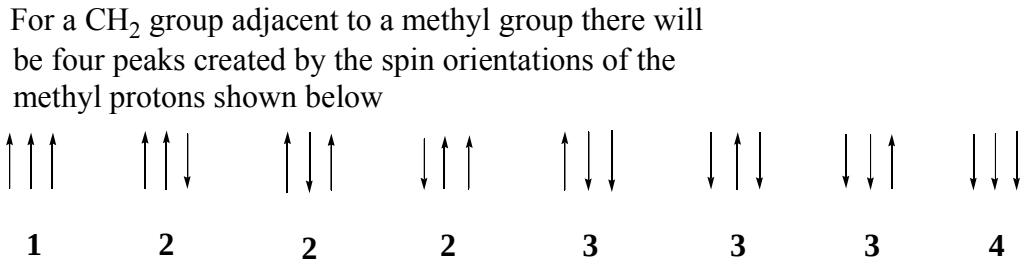


Figure 24. Four different energy levels experienced by a CH_2 group coupled to an adjacent CH_3 group.

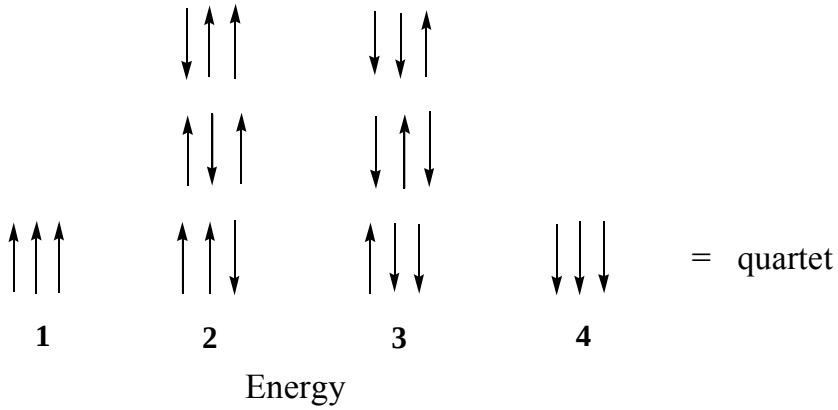


Figure 25. The origin of the quartet signal for methylene protons (CH2) coupled to methyl protons (CH_3).

will contribute in different ways to the magnetic field experienced by the methylene (CH_2) protons (Figure 25). As a results, the total signal for the methylene protons is split into four signals in the intensity ratio of 1:3:3:1. The individual lines in the total signal are separated

by a coupling constant *J*. Spectra of ethyl acetate and 1,3-dichloropropane are given in Figure 26 for illustration. Note the 'roof effect' for signals for coupled protons.

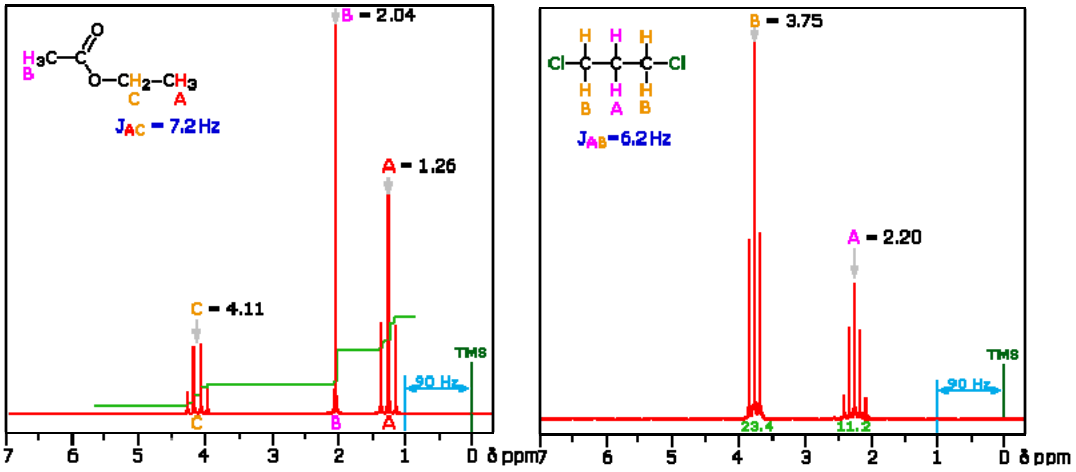


Figure 26. The "roof effect" for coupled protons. Note that the external signals for the multiplets are of lower intensity compared to the intensity of the internal signals deviating from the ideal ratios (see Pascal's triangle, Figure 27).

The following rules summarize the coupling for protons and other spin 1/2 nuclei:

1) Nuclei having the same chemical shift (called *isochronous*) do not exhibit spin-splitting, that is no coupling is seen in the ^{1}H NMR spectrum. They may actually be spin-coupled, but the splitting cannot be observed directly.

2) Nuclei separated by three or fewer bonds (e.g. vicinal and geminal nuclei) will usually be spin-coupled and will show mutual spin-splitting of the resonance signals (same *J*'s), provided they have different chemical shifts. Longer-range coupling may be observed in molecules having rigid configurations of atoms.

3) The magnitude of the observed spin-splitting depends on many factors and is given by the coupling constant *J* (units of Hz). *J* is the same for both partners in a spin-splitting interaction and is independent of the external magnetic field strength.

4) The splitting pattern of a given nucleus (or set of equivalent nuclei) can be predicted by the *n+1* rule, where n is the number of neighboring spin-coupled nuclei with the same (or very similar) Js. If there are 2 neighboring spin-coupled nuclei, the observed signal is a triplet (2 + 1 = 3); if there are three spin-coupled neighbors, the signal is a quartet (3 + 1 = 4). In all cases the central line(s) of the splitting pattern are stronger than those on the periphery (the "roof effect"). The intensity ratio of these lines is given by the numbers in Pascal's triangle (Figure 27). Thus a doublet has 1:1 or equal intensities, a triplet has an intensity ratio of 1:2:1, a quartet 1:3:3:1 etc. (approximate numbers, Figures 26 and 28).

5) If a given nucleus is spin-coupled to two or more sets of neighboring nuclei by different *J* values, the *n+1* rule does not predict the entire splitting pattern. Instead, the splitting due to one *J* set is added to that expected from the other *J* sets (Figure 29).

1

1 1

1 2 1

1 3 3 1

1 4 6 4 1

Figure 27. Pascal's triangle.

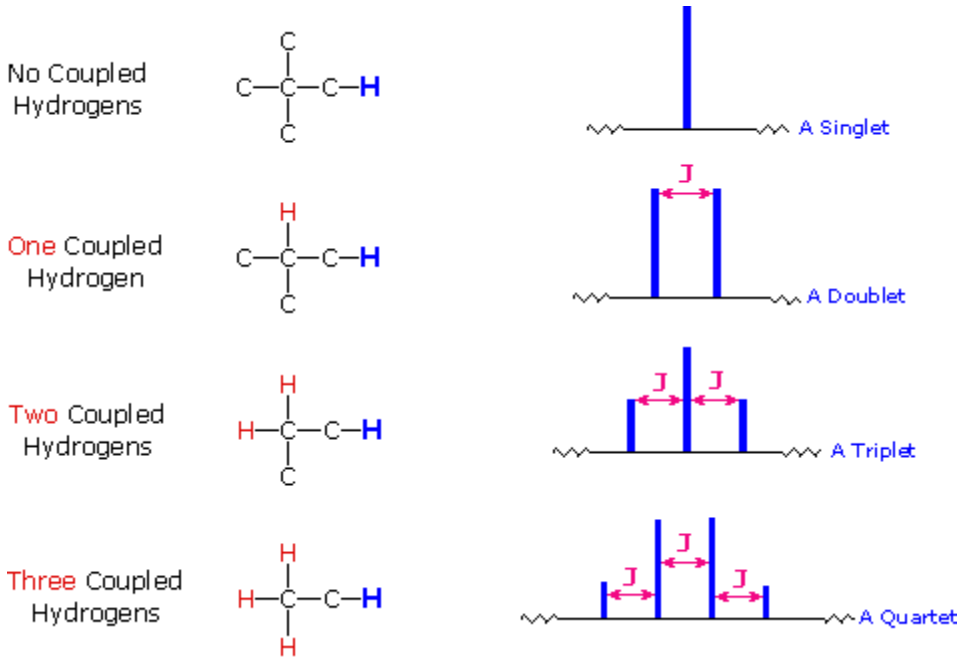


Figure 28. Typical coupling patterns with a single coupling constant *J*.

Typical values of coupling constants are given in Figure 30. The spin-coupling interactions described above for protons may also occur between protons and other spin 1/2 nuclei such as ^{1}H, ^{13}C, ^{19}F, and ^{31}P. If, for example, a ^{19}F atom is spin-coupled to a ^{1}H atom, both nuclei will appear as doublets having the same *J* constant. Spin coupling with nuclei having spin other than 1/2 is more complex and will not be discussed here.

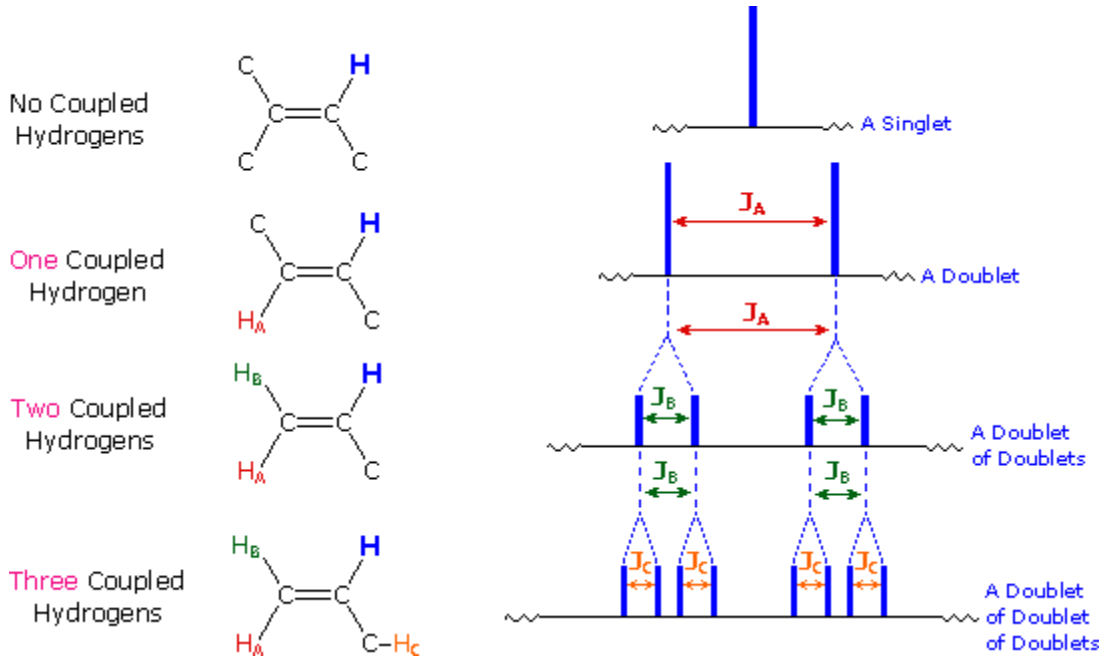


Figure 29. Typical coupling patters with different coupling constants *J*s.

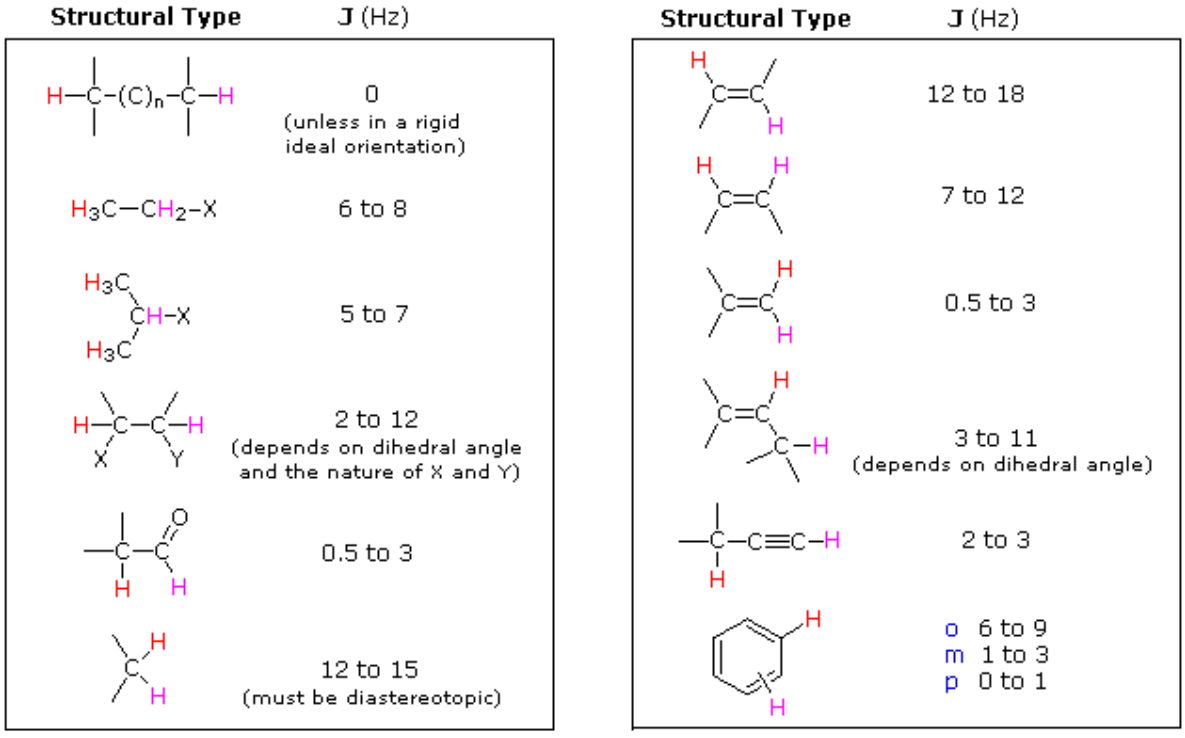
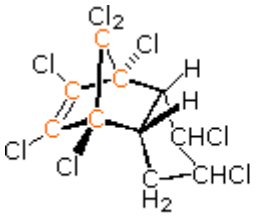
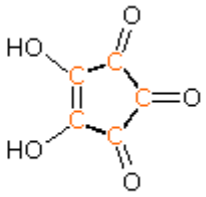


Figure 30. Typical values of coupling constants *J*s (in Hz).

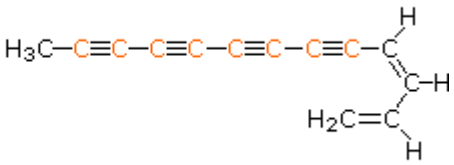
^{13}C NMR spectroscopy. The power and usefulness of ^{1}H NMR spectroscopy as a tool for structural analysis is clearly evident from the discussion above. Unfortunately, when significant portions of a molecule lack C-H bonds, no information is forthcoming. Examples include polychlorinated compounds such as herbicide chlordane, polycarbonyl compounds such as croconic acid, and compounds incorporating triple bonds.



chlordane



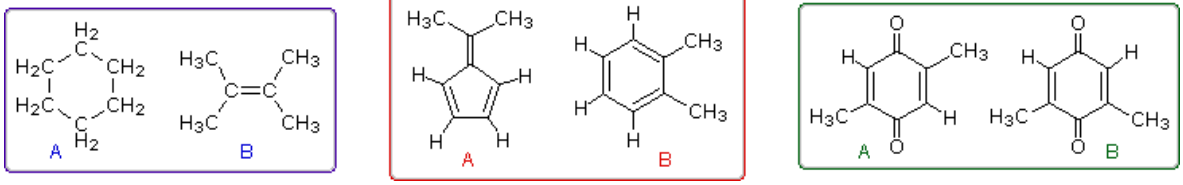
croconic acid



a polyacetylene from *Dahlia*

Even when numerous C-H groups are present, an unambiguous interpretation of a proton NMR spectrum may not be possible. The following diagram depicts three pairs of isomers (A & B) which display similar proton NMR spectra. Although a careful determination of chemical shifts should permit the first pair of compounds to be distinguished, the second and third cases might be difficult to identify by proton NMR alone.

1 **2** **3**



These difficulties would be largely resolved if the carbon atoms of a molecule could be probed by NMR in the same fashion as the hydrogen atoms. Since the major isotope of carbon ^{12}C is non-magnetic, this option seems unrealistic. Fortunately, 1.1% of elemental carbon is the ^{13}C isotope, which has a spin I = 1/2, so in principle it should be possible to conduct a carbon NMR experiment. It is worth noting here, that if much higher abundances of ^{13}C were naturally present in all carbon compounds, proton NMR would become much more complicated due to large one-bond coupling of ^{13}C and ^{1}H.

The most important operational technique that has led to successful and routine ^{13}C NMR spectroscopy is the use of high-field pulse technology coupled with broad-band heteronuclear decoupling of all protons. For the decoupling, the sample is irradiated with a relatively intense range of frequencies that correspond to precessional frequencies of all protons in the molecule. As a result, these protons become saturated, no further absorption of the irradiation energy is possible, and the protons are no longer coupled to ^{13}C nuclei. The results of repeated

pulse sequences are accumulated to provide improved signal strength. When acquired in this manner, the carbon NMR spectrum of a compound displays a single sharp signal for each structurally distinct carbon atom in a molecule because the proton couplings have been removed and the probability of carbon-carbon coupling is low due to the low natural abundance of ^{13}C. More specifically, there is low probability for two ^{13}C nuclei to be adjacent to each other in a molecule. The spectrum of camphor, shown in Figure 31, is illustrative. Furthermore, a comparison with the ^{1}H NMR spectrum on the right illustrates some of the advantageous characteristics of carbon NMR. The range of ^{13}C chemical shifts is nearly twenty times greater than that for protons, and this together with the lack of signal splitting makes it more likely that every structurally distinct carbon atom will produce a separate signal. The only clearly identifiable signals in the proton spectrum are those from the methyl groups. The remaining protons have resonance signals between 1.0 and 2.8 ppm from TMS, and they overlap badly thanks to spin-spin splitting.

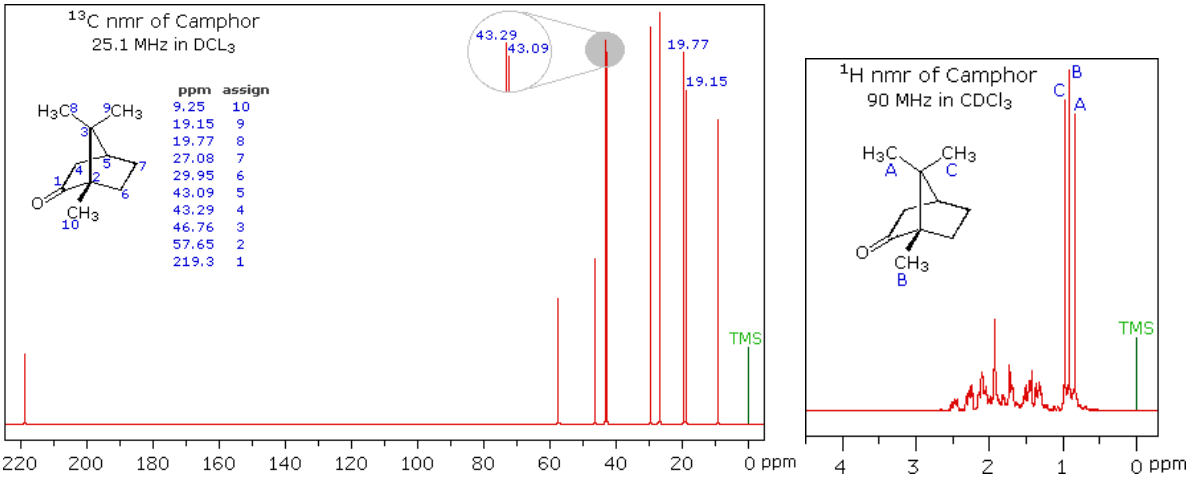


Figure 31. ^{13}C and ^{1}H NMR spectra of camphor.

Unlike in proton NMR spectroscopy, *the relative strengths of carbon NMR signals are not normally proportional to the number of ^{13}C atoms generating these signals*. As a result, the number of discrete signals and their chemical shifts are the most important pieces of evidence delivered by a carbon spectrum. The general distribution of carbon chemical shifts associated with different functional groups is summarized in Figure 32. Note that the over 200 ppm range of chemical shifts shown here is much greater than that observed for proton chemical shifts.

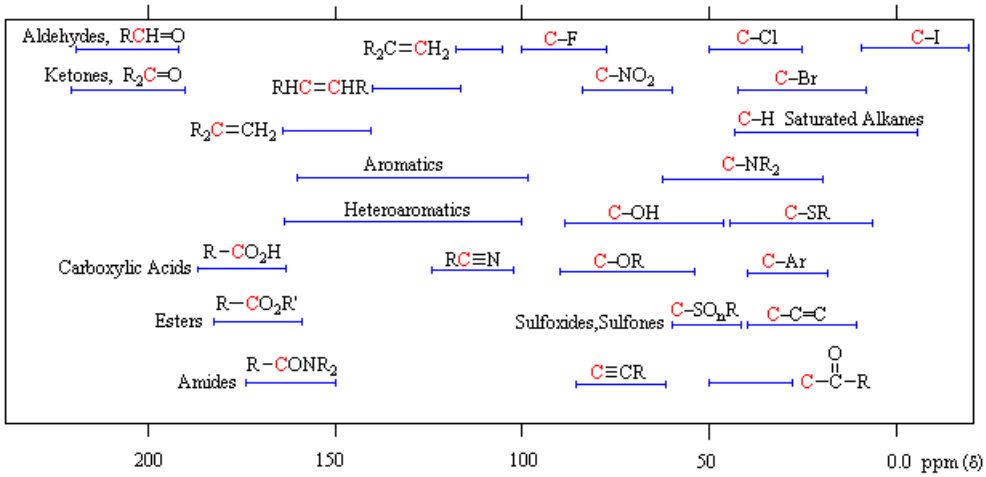
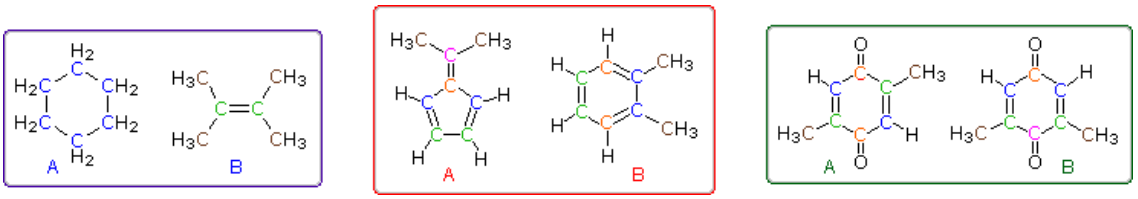


Figure 32. ^{13}C NMR chemical shifts for various classes of compounds. The δ scale is relative to TMS at $\delta = 0$.

The isomeric pairs previously examined as giving very similar proton NMR spectra can be distinguished by carbon NMR spectroscopy. In the first example, cyclohexane (A) and 2,3-dimethyl-2-butene (B) both give a single sharp resonance signal in the proton NMR spectra (the former at δ 1.43 and the latter at δ 1.64). However, the ^{13}C NMR spectrum of cyclohexane displays a single signal at δ 27.1, generated by the equivalent ring carbon atoms; whereas the ^{13}C NMR spectrum of the isomeric alkene (B) shows two signals, one at δ 20.4 (typical for a sp^3 carbon) from the methyl carbons and the other at δ 123.5 ppm (typical for a sp^2 carbon).



The C_8H_{10} isomers in the center have pairs of homotopic (structurally identical) carbon and hydrogen atoms, so symmetry should simplify their NMR spectra. The fulvene (isomer A) has five structurally different groups of carbon atoms and should display five ^{13}C NMR signals (one near 20 ppm and the other four greater than 100 ppm). Although *ortho*-xylene (isomer B) will have a proton NMR spectrum very similar to that of isomer A, it should only display four ^{13}C nmr signals, originating from the four different groups of carbon atoms. The methyl carbon signal will appear at high field (near 20 ppm), and the aromatic ring carbons will all give signals having $\delta > 100$. Finally, the last isomeric pair, quinones A and B in the green box, are easily distinguished by carbon NMR. Isomer A displays only four carbon NMR signals (δ 15.4, 133.4, 145.8, and 187.9); whereas, isomer B displays five signals (δ 15.9, 133.3, 145.8, 187.5, and 188.1), the additional signal coming from the non-identity of the two carbonyl carbons.

Reporting the spectral and analytical data. All major synthetic journals have adopted the following format for characterization of new compounds (or closely similar one), and it should be used in the students' reports. Compound in Example 7, the section High-resolution mass spectrometry (HR-MS), serves as an example.

2,6-Bis[2'-[1''-butyl-6''-chlorobenz[*cd*]indol-2''(1''*H*)ylidene]ethylidene]cyclohexanone: mp > 116 ^{o}C (dec.); ^{1}H NMR (400 MHz, CD_2Cl_2): δ 0.97 (t, J = 7.5 Hz, 6H), 1.44 (m, 4H), 1.79 (quint, J = 7.5 Hz, 4H), 1.95 (quint, J = 5.5 Hz, 2H), 2.79 (t, J = 5.5 Hz, 4H), 3.78 (t, J = 7.5 Hz, 2H), 3.92 (t, J = 7.5 Hz, 2H), 6.11 (br d, J = 13 Hz, 2H), 6.55 (d, J = 8 Hz, 2H), 7.39 (d, J = 8 Hz, 2H), 7.75 (t, J = 8 Hz, 2H), 7.93 (d, J = 8 Hz, 2H), 8.22 (d, J = 8 Hz, 2H), 8.51 (br d, J = 13 Hz, 2H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 14.1, 14.2, 20.8, 20.9, 23.0, 26.8, 30.3, 30.5, 31.3, 40.6, 43.2, 101.6, 101.9, 102.2, 105.9, 120.7, 123.6, 123.8, 124.5, 125.0, 125.2, 126.7, 127.7, 128.1, 128.41, 128.44, 128.5, 128.8, 129.3, 129.7, 130.0, 130.2, 131.8, 132.5, 133.1, 139.5, 143.4, 146.9, 167.9, 186.7; HR-MS (FAB): Calcd for $C_{40}H_{39}(^{35}Cl)_2N_2O$ m/z 633.2439, obsd m/z 633.2456 (2.6 ppm error). Analysis for a sample dried at 25 ^{o}C/0.1 mmHg for 3 days. Calcd for $C_{40}H_{39}Cl_2N_2O•1/2H_2O$: C, 74.76; H, 6.12; N, 4.36. Found: C, 75.17; H, 6.30; N, 4.53 (presence of water was evident from the IR spectrum: ν 3450 cm^{-1}).

Note that the multiplicities of signals in the ^{1}H NMR spectrum are abbreviated as d (doublet), br d (broad doublet), t (triplet), quint (quintet), and m (multiplet without defined coupling constants). The ^{1}H NMR chemical shifts are reported to two decimal places. For pairs of coupled protons the coupling constant J is reported twice for each coupled proton. It is customary to report ^{13}C NMR chemical shifts to one decimal place. The structure of this compound given in the Example 7 above is not consistent with the ^{13}C NMR spectrum.[7] More specifically, the symmetry implies 21 signals in the spectrum instead of the 40 signals found experimentally, one signal for each carbon atom in the molecule. This apparent discrepancy can be explained in terms of the *anti* conformation of the molecule with the terminal heterocyclic subunits positioned *anti* to each other around the central cyclohexanone subunit. Can you draw this conformation? Note that the presence of crystallization water ($2M•H_2O$ in the crystal unit) cannot be found by mass spectrometry. The presence of water is often visualized in the ^{1}H NMR spectra taken in $CDCl_3$ or CD_2Cl_2 solution. Two singlets that can be seen in the range of δ 0.5 – 2.0 correspond to a monomer H_2O and a hydrogen-bonded dimer $(H_2O)_2$. However, these signals are often due to the presence of water in the solvent. Storing the NMR solvents in a tightly closed bottle with activated molecular sieves 3A or 4A (a few pellets) efficiently removes water.

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PHASE-TRANSFER CATALYSIS (PTC)

The fundamental problem of organic synthesis is to find conditions for the efficient interaction between the molecules of substrates. If one reactant is a polar inorganic material such as a salt, the reaction in a non-polar organic solvent would be heterogeneous, that is, inefficient. Most of the organic compounds are soluble in non-polar organic solvents in which inorganic reagents are not soluble. Attempts have been made to modify conditions for the reactions of incompatible substrates by using solvents that are miscible with each other and which dissolve separately individual starting materials. The most common solvents of this type are aqueous alcohols. Unfortunately, both water and alcohols strongly deactivate polar inorganic compounds by solvation through hydrogen bonding and, as a result, the inorganic molecules are shielded and interact inefficiently with organic substrates. A better solution to the reactivity problem is the use of polar aprotic solvents exemplified by DMSO, DMF, and NMP (Figure 1). They are polar because of charge separation as shown, and they are aprotic

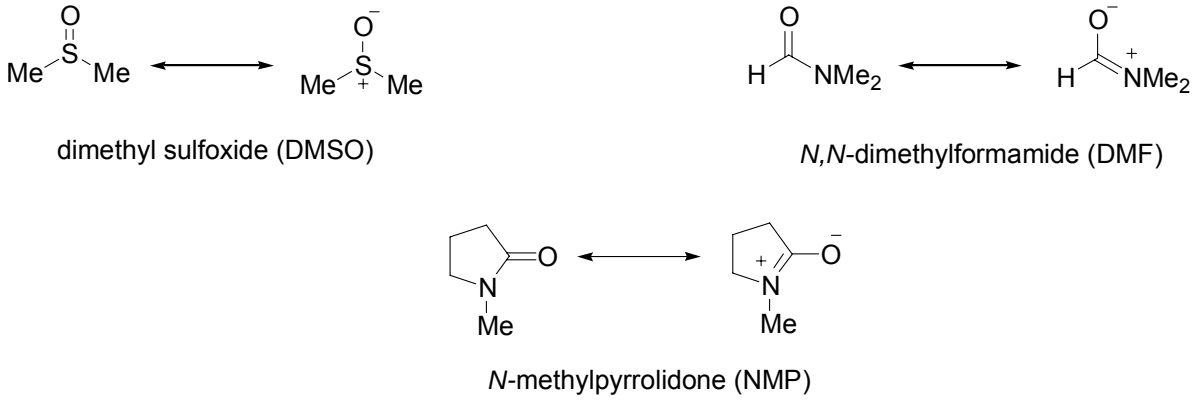
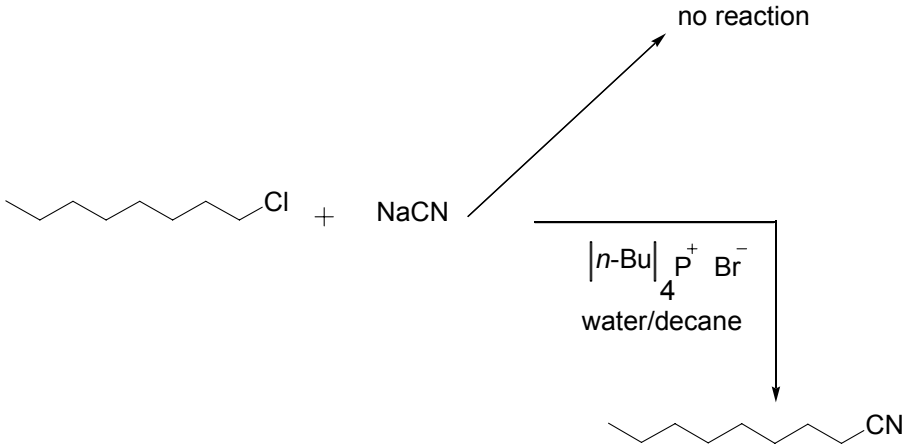


Figure 1. Examples of polar aprotic solvents.

because of high pK_a and inability to act as a hydrogen donor for hydrogen bonding interaction. The polar aprotic solvents dissolve organic compounds because they are organic solvents and they dissolve inorganic salts because of their high polarity. Thus the reactions between organic and inorganic substrates proceed in a homogeneous phase and are relatively fast. The drawback of using polar aprotic solvents is their high cost and difficulty of workup due to their high boiling point. Addition of solvent to any chemical process increases the expense of the reaction. First, there is the cost of obtaining the solvent and second, there is a cost in time and energy of removing it to purify the product. Further, addition of any solvent reduces the effective concentration of the reagents and, accordingly, the rate of a bimolecular process decreases as the concentration decreases. An ingenious way to conduct many reactions using water, the most inexpensive solvent, was introduced for the first time in 1965 by Polish chemist Makosza[1] followed by synthetic studies in 1971 by American chemist Starks.[2] They showed that many incompatible substrates may be forced to react by using a mixture of water (dissolving polar compounds) and a common inexpensive organic solvent (dissolving non-polar compounds) that is not miscible in water, in the presence of a small amount of so called phase-transfer catalyst. The principle of the phase transfer catalysis is explained in Scheme 1 and Figure 2 by using the S_N2 reaction of octyl chloride with sodium

Scheme 1



cyanide. Under neat conditions (no solvent) or in a solution of octyl chloride in decane the reaction is extremely slow and inefficient (low yield) because sodium cyanide is not soluble under either conditions. Thus, no product was observed after more than a week at reflux temperature. By contrast, octyl cyanide, the desired product, is formed quickly and efficiently when a mixture of octyl chloride, sodium cyanide, water, decane, and a catalytic amount of tetrabutylphosphonium bromide (abbreviated as $Bu_4P^+Br^-$, a phase transfer catalyst) is stirred

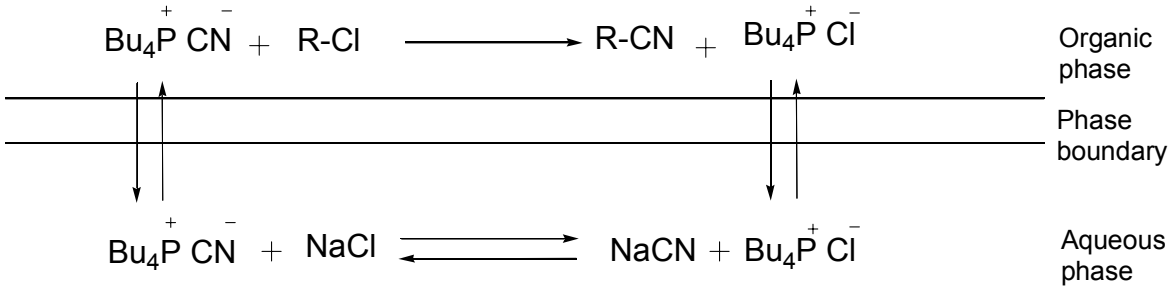


Figure 2. The principle of phase-transfer catalysis developed by Makosza

rapidly to form a thick emulsion. The stirring greatly increases the surface area between water and decane. Initially, the aqueous layer obviously contains sodium cyanide, and octyl chloride is present in the organic phase. What about the catalyst? It contains highly lipophilic butyl groups, which makes the compound soluble in an organic solvent. It is also a polar phosphonium salt, which makes the compound soluble in water as well. Thus, the catalyst can travel easily from the aqueous phase to the organic phase and back (Figure 2). When an anion exchange process takes place in an aqueous phase, this leads to formation of tetrabutylphosphonium cyanide ($Bu_4P^+CN^-$). The latter is soluble in the organic phase, where the reaction of octyl chloride with cyanide anion takes place. The byproduct of this reaction in the organic phase is the quaternary phosphonium chloride ($Bu_4P^+Cl^-$) which returns to the aqueous phase and can then exchange with another anion. Thus, the process is catalytic. In

some cases the catalytic reaction takes place at the interface of the organic and aqueous phases. The mechanism (Figure 2) was initially proposed by Makosza and further developed by Liotta (Georgia Tech, Atlanta). The high reactivity of nucleophiles under phase transfer catalysis was attributed to their poor solvation in the organic phase or at the phase boundary. The poorly solvated nucleophiles were sometimes called 'naked' or 'bare', although neither designation is really appropriate. Indeed, Makosza suggested that the anions should be called 'bikini' ions because each ion pair is associated with a few molecules of water, or partly covered by water, especially when extracted from an aqueous solution. Typical phase-transfer catalysts are shown in Figure 3. These are ammonium derivatives **1 – 4**, phosphonium salts **5** and **6**, crown ethers **7 – 9**, and a cryptand **10**. The cyclic ethers **7 – 10** efficiently complex metal ion, especially potassium cation. The potassium complexes with **7** and **10** are shown in Figure 3 for illustration. The metal ion complexation leaves the anion (nucleophile or base)

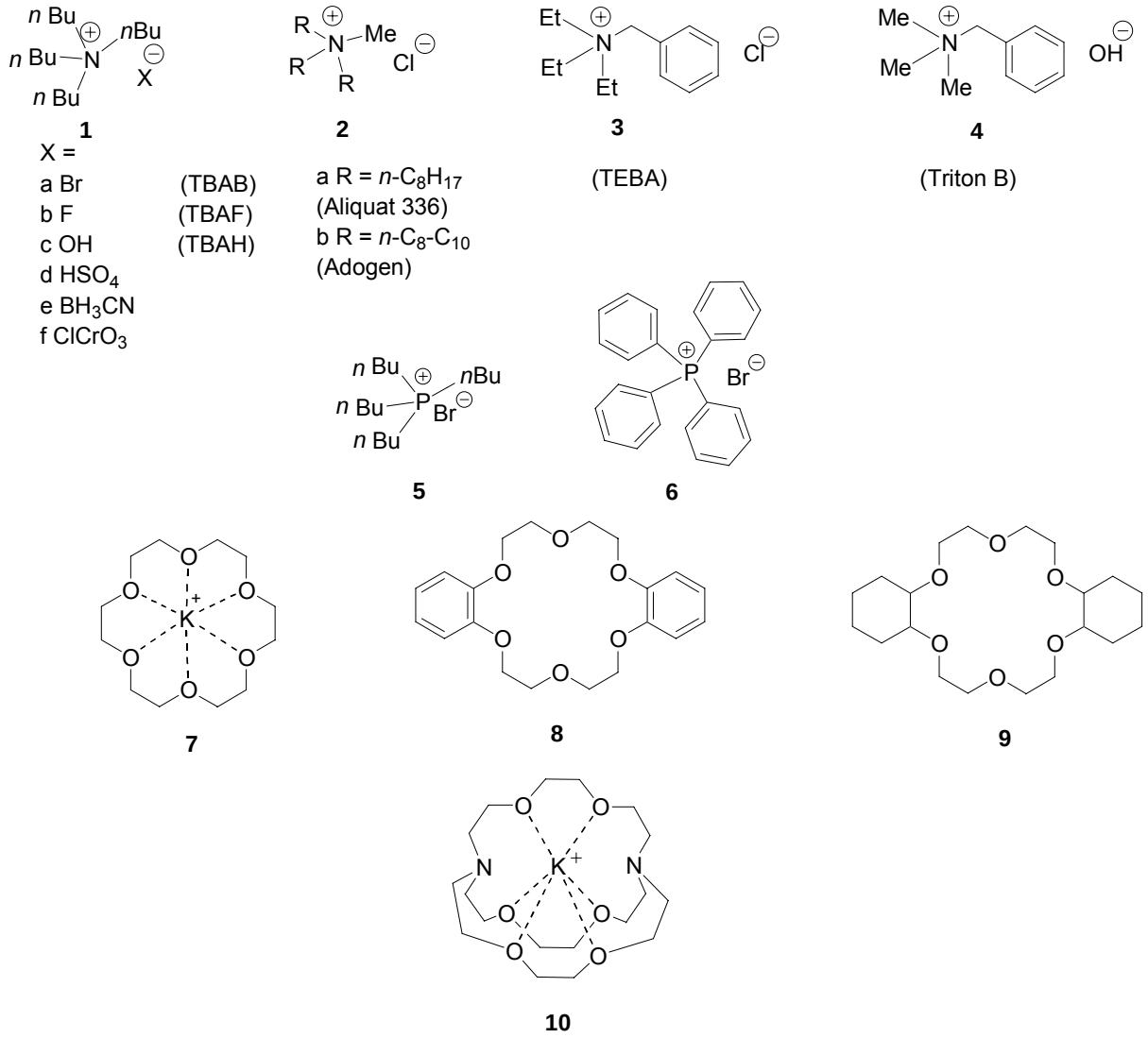
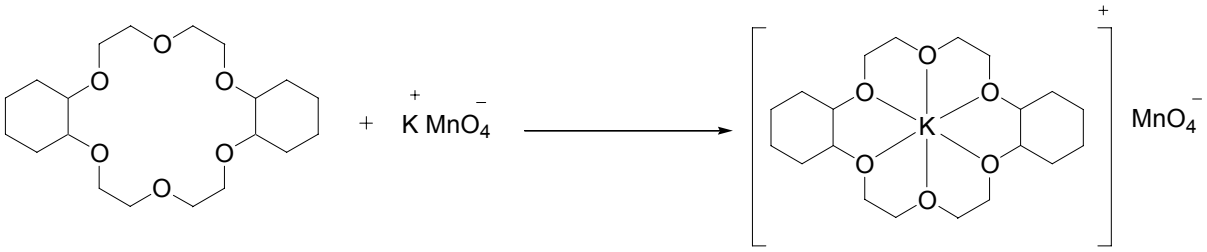


Figure 3. Typical phase transfer catalysts: ammonium **1 – 4**, phosphonium **5**, **6**, crown ether **7 – 9**, and cryptand **10** derivatives. Complexes with potassium cation are shown for **7** and **10**.

only loosely interacting with the metal cation, thereby enhancing reactivity of the 'bikini'anion. All compounds shown in Figure 3 are commercially available.[3] The cyclic ethers **7** – **10** are the most efficient phase-transfer catalysts, albeit quite expensive. They can also be used to dissolve organic and inorganic alkali metal salts in organic solvent. The crown ether complexes the cation and provides it with an organic lipophilic exterior so that the complex is soluble in organic solvents. The anion is carried along into organic solution as part of the ion pair. For example, potassium permanganate ($KMnO_4$) complexed to the crown ether **9** (Figure 3) is soluble in benzene and the solution is known as "purple benzene" (see the Equation below). It is useful in various oxidation reactions.

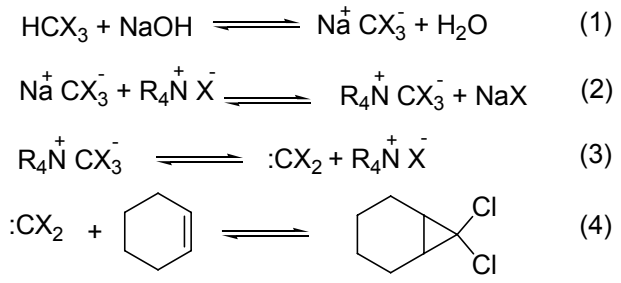


Most PTC reactions are conducted in the presence of inexpensive ammonium compounds **1** – **4**. Note that all ammonium catalysts contain at least 13 carbon atoms in the molecule. Molecules with fewer number of carbon atoms are not lipophilic enough for a good solubility of the cationic derivative in organic solvents. Synthesis of 7,7-dichloronorcarane (this experiment) is conducted in the presence of catalyst **3**. The preparation of a nitrile discussed above and synthesis of 7,7-dichloronorcarane are two examples selected from a vast list of other reactions conducted efficiently under PCT conditions. Additional examples are Williamson ether synthesis, $KMnO_4$ mediated oxidation of alkenes to 1,2-glycols, various alkylation reactions, and Wittig reaction, to name a few.[4-7]

Synthesis of 7,7-dichloronorcarane[4,5] under PTC conditions

A carbene (X_2C:) is a neutral molecule containing a divalent carbon atom that has only six electrons in its valence shell instead of the stable configuration of eight. Thus, carbene is electron-deficient and behaves as an electrophile. It reacts with an electron-rich carbon-carbon double bond in one step reaction without intermediate formation. Dihalocyclopropanes are generally prepared by the addition of dihalocarbenes to alkenes. For example, 7,7-dichloronorcarane (eq. 4) is prepared by the addition reaction of dichlorocarbene to cyclohexene. Dichlorocarbene can be generated by the reaction of chloroform with a strong base. Traditionally, the reaction has been carried out in a homogeneous phase in anhydrous alcohol solvent using alkoxide base. Typical systems are MeOH/MeONa and t-BuOH/t-BuOK. Unfortunately, this technique is experimentally difficult because the reaction must be conducted under anhydrous conditions. In the presence of water, intermediate dichlorocarbene is quickly hydrolyzed to carbon monoxide and hydrogen chloride. A two-phase reaction conducted in the presence of a phase-transfer catalyst is a convenient alternative to the homogeneous reaction discussed above. Chloroform is both a solvent and a precursor to dichlorocarbene in the reaction with aqueous sodium hydroxide conducted in the presence of

TEBA (**3** in Figure 3) as a phase transfer catalyst. The synthesis is explained in Equations 1 -4 and in Figure 4.



7,7-dichloronorcarane
(7,7-dichlorobicyclo[4.1.0]heptane)

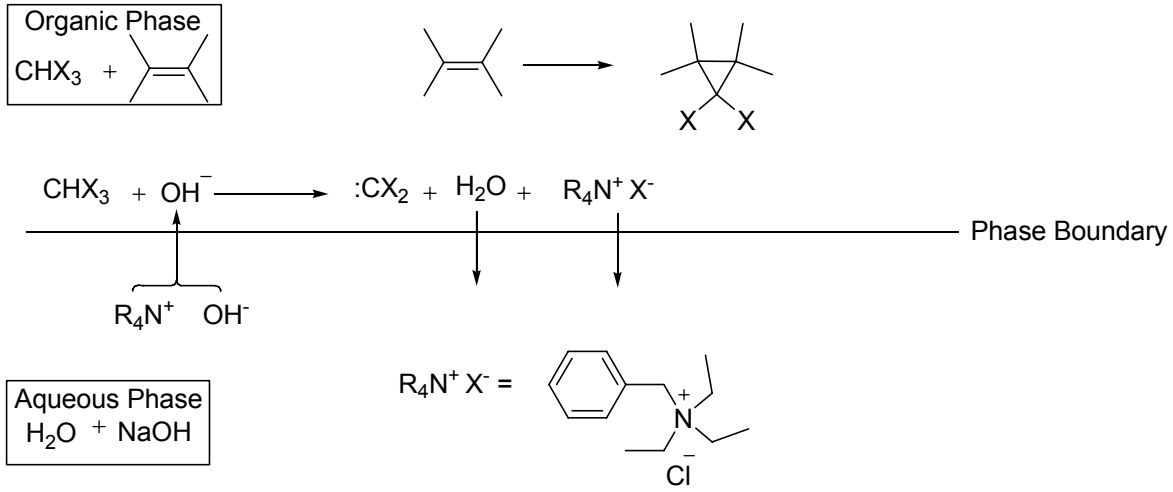


Figure 4. The mechanism of the norcarane synthesis

Chloroform: Density 1.49 g/ml, solvent and reagent
Sodium hydroxide (NaOH): base
Cyclohexene: Density 0.80 g/ml, reagent
Benzyltriethylammonium chloride (TEBA): phase-transfer catalyst
Dichlorometane: solvent for workup

Techniques: Generation of a carbene, phase-transfer catalysis, distillation, IR, GC-MS, ^{1}H-NMR.

The experimental procedure

Dissolve sodium hydroxide (15 g) in water (15 ml) in 500-mL Erlenmeyer flask to prepare a 50% aqueous solution of sodium hydroxide. Place a magnetic stirring bar in the flask and swirl the mixture on a magnetic stirrer to help dissolve the solid. After the sodium hydroxide has dissolved, cool the solution to 20 ^{0}C with the aid of an ice bath. In a 100-ml Erlenmeyer

flask, place cyclohexene (10 ml) and chloroform (25 ml), and swirl the flask to mix the liquids. **Caution**: *All these operations should be conducted in a hood.*
Weigh out benzyltriethylammonium chloride (TEBA, 1.0 g) on a smooth piece of paper and reclose the bottle immediately (it is hygroscopic). **Caution**: *Avoid contact with skin*. Transfer the catalyst to the 500-mL flask, and immediately add the cyclohexene-choroform mixture. Stir the mixture as rapidly as possible on a magnetic stirrer for 30 min to ensure adequate mixing. As the mixture is swirled, a thick emulsion will form and the temperature will rise (not to exceed 50 ^{0}C). Check the temperature periodically, but do not leave the thermometer in the flask. Allow the mixture to return to room temperature, with occasional swirling, over an additional 30 to 45 min and without external cooling. Then water (75 ml) is added to the mixture along with dichloromethane (25 ml). This mixture is then added to a 125-ml separatory funnel and shaken vigorously. The organic layer (lower layer) is drained and the aqueous layer is extracted with another portion of dichloromethane (25 ml) again. The organic layers are combined, washed with a saturated solution of sodium chloride (30 ml), and then dried with anhydrous magnesium sulfate or sodium sulfate (~ 2 g). Now gravity filter the solution to remove the salt. The dried organic layer is concentrated on a rotary evaporator to remove excess of dichloromethane. The residue is distilled on a Kugelrohr under reduced pressure (b.p. 120-130 ^{0}C/20 mmHg) to give colorless oil (11 g, 58%).

Post-synthetic assignments

1. Calculate the yield of your preparation.
2. Record boiling point, IR, mass spectra (GC-MS) and ^{1}H NMR of the product. Correlate the spectra with the structure. Correlate the presence of two chlorine atoms in the molecule with the molecular ion peaks in the mass spectrum.
3. Draw the mechanism for the generation of dichlorocarbene.
4. Explain the IUPAC name of 7,7-dichloronorcarane – see Equation 4.
5. A boiling point of a liquid is 160 ^{0}C/10 mmHg. What would be the b.p. at 1 mmHg?
6. Explain why tetraethylammonium chloride is a poor phase-transfer catalyst.

Phase transfer catalysis: references

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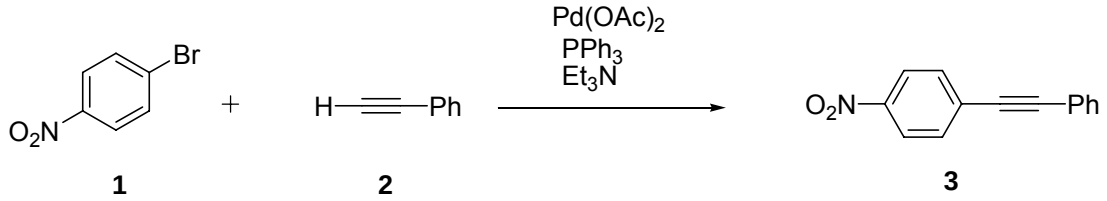
PALLADIUM-MEDIATED COUPLING REACTIONS

Synthesis of 4-nitrodiphenylacetylene (3)



1 **2** **3**

4-Bromonitrobenzene (1): Mp 124-126 ^{o}C
Phenylacetylene or phenylethyne (2): Density 0.93 g/ml, bp 142-144 ^{o}C
Palladium acetate
Triphenylphosphine: Mp 79-81 ^{o}C
Triethylamine, hydrochloric acid (2M), **ethanol**

Techniques: Sublimation, crystallization, mp, IR, ^{1}H NMR and ^{13}C NMR spectroscopy, GC-MS

4-Bromonitrobenzene (2.02 g 10 mmol), phenylacetylene (1.53 g, 1.65 ml, 15 mmol), palladium acetate (2.8 mg, 0.12 mol%), triphenylphosphine (6.6 mg, 0.25 mol%), and triethylamine (20 ml) are added to a 100-ml two-neck flask equipped with a reflux condenser and a magnetic stirring bar. The setup is gently flushed with nitrogen by inserting a nitrogen tubing to the top of the condenser, then the remaining neck of the flask is closed with a glass stopper, the nitrogen tubing is removed from the condenser which immediately is closed with a rubber septum. A needle connected to the nitrogen line kept under normal pressure is introduced to the septum, in order to keep the nitrogen atmosphere in the setup under normal pressure. The solution is stirred and heated gently to 100 ^{0}C for 75 min. The initial exothermic reaction will cause vigorous boiling but it will subside as the process nears completion. After cooling, the mixture is treated with hydrochloric acid (2M, 40 ml) and the resultant precipitate of product **3** is collected by filtration and dried by leaving the wet material on the open air until the next lab period or under a reduced pressure The crude product **3** is sublimed (115 ^{o}C/2-3 mmHg). For final purification, the sublimed product is dissolved in a minimum amount of warm ethanol and the solution is gently diluted with a two-fold volume of warm water. After cooling, the crystalline material is dried under a reduced pressure.

Post-synthetic assignments

1. Calculate the yield.
2. Record IR, ^{1}H NMR, ^{13}C NMR, and mass spectra (GC-MS) of product **3**. Correlate the spectra with the structure.

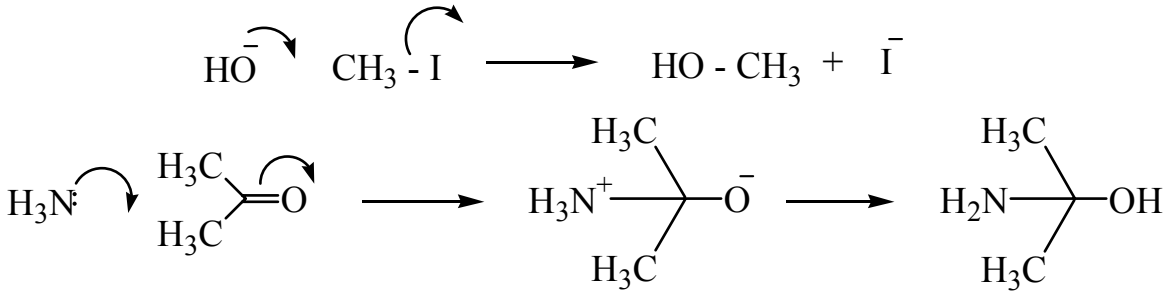
For a comprehensive account of palladium in organic synthesis, including full experimental details, see '*Palladium Reagents in Organic Synthesis*' by R.F. Heck, Academic Press, 1985, p. 300

ORGANOMAGNESIUM (GRIGNARD) AND ORGANOLITHIUM REAGENTS

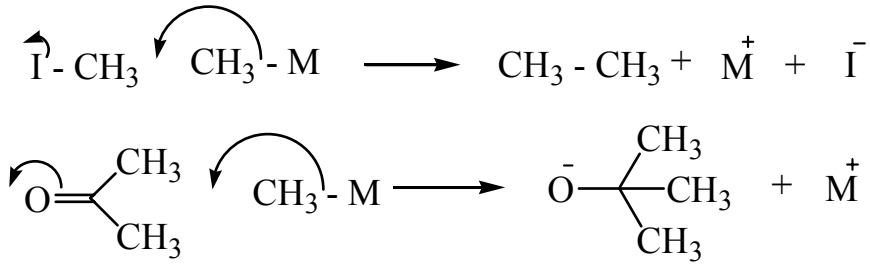
General

Typical organometallic reagents contain a carbon-metal bond. All the non-metallic elements to which carbon is commonly bonded are more electronegative than carbon. As a consequence, the carbon atom is positively polarized and is susceptible to attack by nucleophiles (Scheme 1). By contrast, carbon bonded to a metal (M, electropositive element) is negatively polarized and is susceptible to attack by electrophiles (Scheme 2). The organic

Scheme 1



Scheme 2



compounds of potassium and sodium are insoluble in non-polar solvents. Derivatives of less electropositive metals such as lithium and magnesium are essentially covalent and are soluble in ether solvents including diethyl ether and tetrahydrofuran. The reactivity of organometallics can be correlated with their ionic character (Figure 1). Derivatives of potassium and sodium are by far the most reactive. For example, they ignite spontaneously in air. Organomagnesium (Grignard) reagents are least reactive in the series and they react with oxygen less vigorously. Lithium compounds are more reactive than magnesium counterparts.

Metal	K	Na	Li	Mg
% Ionic Character	51	47	43	35

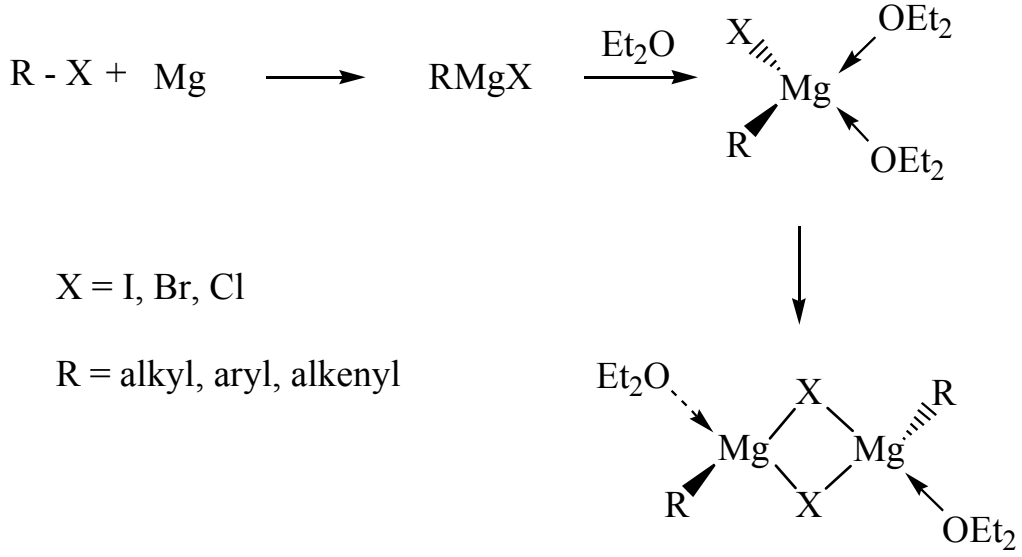
reactivity of organometallic reagents

Figure 1. Ionic character and reactivity of reagents containing carbon – metal function.

Grignard reagents[1,6]

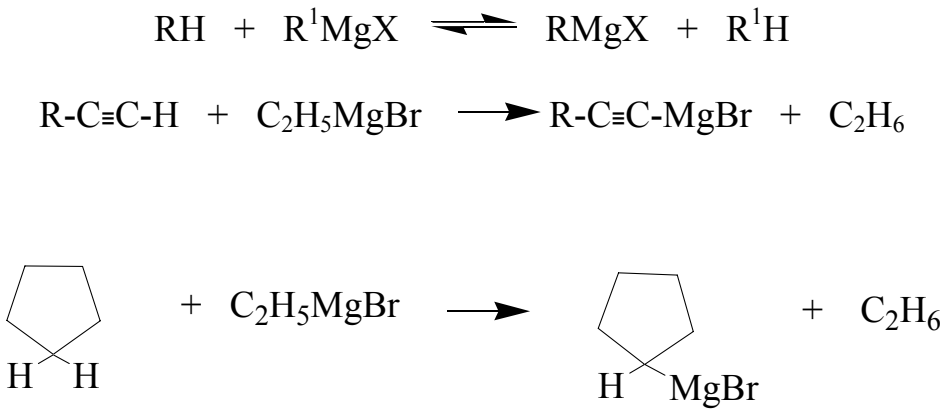
These reagents are prepared and used in solution in an ether solvent, in which they exist in solution as monomeric and dimeric coordination complexes (Scheme 3). The Grignard reagents are not soluble in hydrocarbons and this is why they are generated and used in ether solvents exclusively. The standard method for the preparation of a Grignard reagent is the reaction of an organic halide in dry diethyl ether with magnesium metal (as turnings, small granules or powder). Thus generated reagent is not isolated from solution but is used directly for the required synthesis. The group R may be alkyl, aryl, or alkenyl. Amongst the halides, the order of reactivity with metallic magnesium is I > Br > Cl, and organomagnesium fluorides (RMgF) have not been prepared. Since methyl chloride (CH_3Cl) and methyl bromide (CH_3Br) are gases at room temperature, methyl iodide (CH_3I, bp 43 ^oC) is generally used to generate CH_3MgBr. Likewise, liquid ethyl bromide (C_2H_5Br) is preferred to gaseous ethyl chloride (C_2H_5Cl) for the ethyl Grignard reagent.

Scheme 3



All operations with Grignard reagents must be conducted in rigorously dry ether solvents. Grignard reagents react with oxygen, and it is helpful, although not usually essential, to exclude air for the reactions conducted in diethyl ether. During the formation of the reagent, air is largely kept out by the blanket of ether vapor above the refuxing solution and the reagent is normally used straightaway. Alkyl and aryl halides, except aryl chlorides, normally react readily with magnesium metal in anhydrous diethyl ether (abbreviated as *ether* or Et_2O). Aryl chlorides react in anhydrous tetrahydrofuran (THF). With some halides it is necessary to add a small crystal of iodine in order to activate the surface of the metal. Allyl halides react readily in ether, but since they also react readily with Grignard reagents (by coupling reaction), it is important to keep the concentration of the halide to a minimum in the presence of the Grignard reagent. This is done by the slow addition of a dilute solution of the allyl halide to a large excess of a vigorously stirred suspension of magnesium metal in ether. Vinyl halides do not react in ether but do so in THF.

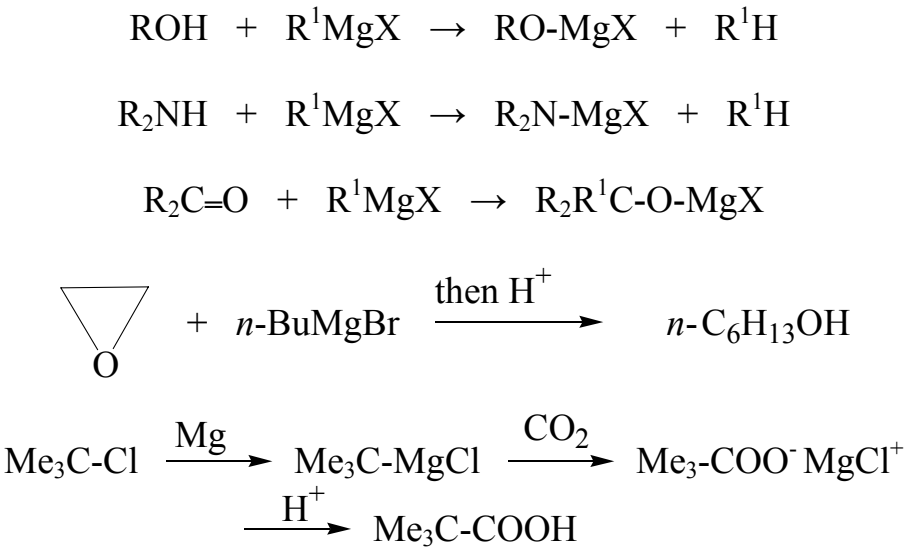
Scheme 4



Grignard reagents can also be generated by metallation (hydrogen – metal exchange) using a preformed Grignard reagent (Scheme 4). The method is only suitable when the carbon atom attached to magnesium in the product (reagent to be prepared) is markedly more electronegative than the carbon atom in the starting Grignard compound (hybridization C-sp^3). Alkynyl Grignards (C-sp)are normally prepared by this method and so are those from other acidic hydrocarbons such as cyclopentadiene (C-sp^2 in the product).

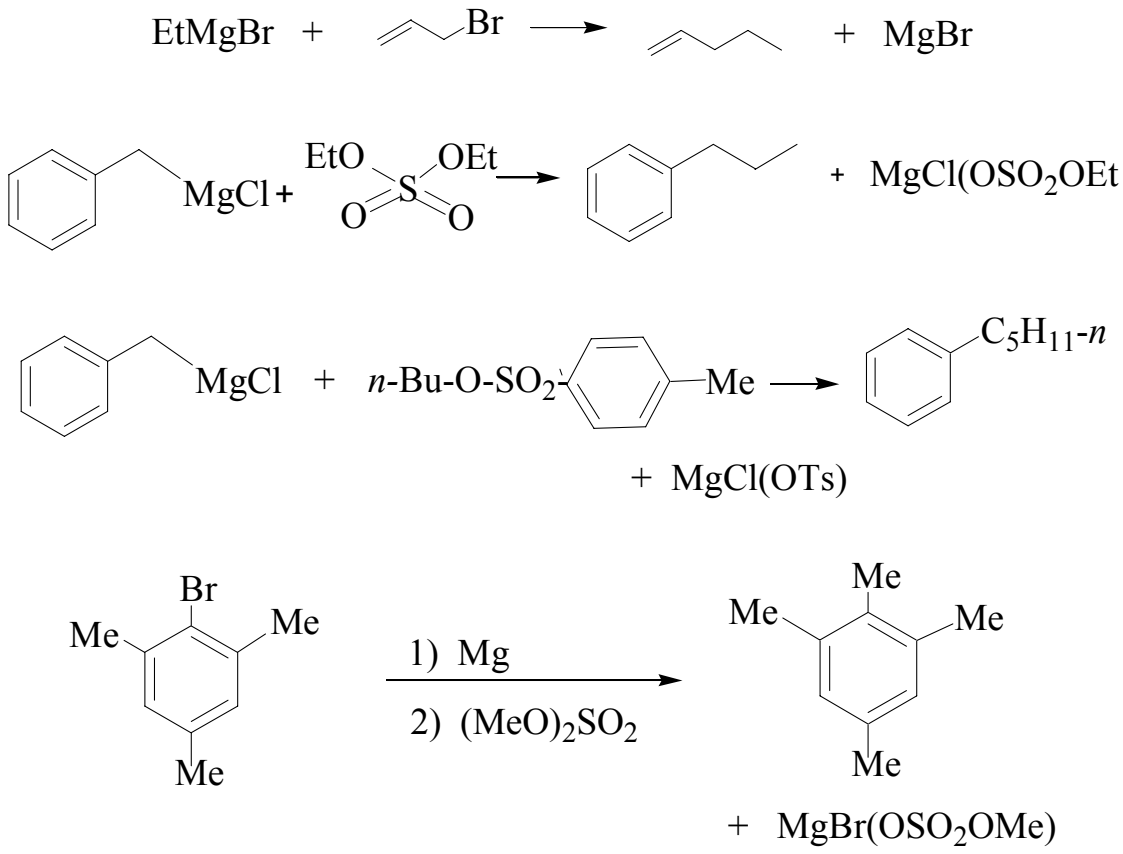
A useful working guide to the mode of reaction of Grignard reagents is that the direction of reaction is such that the magnesium atom is transferred to a more electronegative atom, as in metallation procedure for their preparation. All OH- and NH-containing compounds react by replacement of hydrogen. In the reactions at carbon centers, the magnesium is similarly transferred to oxygen or nitrogen when one of these elements is present (Scheme 5).

Scheme 5



Although saturated alkyl Grignard reagents and phenyl Grignard reagents react with saturated alkyl halides in the S_N2 manner, only the reaction with methyl halides is of practical value. The reaction with other saturated alkyl halides is slow and the yield are low. On the other hand, allyl and benzyl halides (which are more reactive than alkyl halides in S_N2 reactions) react efficiently (Scheme 6). Alkyl compounds containing better leaving groups than the halides, such as alkyl sulfates and sulfonates, react in much higher yields than the alkyl halides. Examples in Scheme 6 include the synthesis of propylbenzene (yield 75%) by the reaction of benzyl magnesium bromide and diethyl sulfate, the synthesis of pentylbenzene (yield 60%) by the reaction of benzyl magnesium chloride and butyl *para*-toluenesulfonate, and the efficient preparation of isodurene (1,2,3,5-tetramethylbenzene, a sterically congested molecule) from mesityl bromide and dimethyl sulfate (yield 60%).

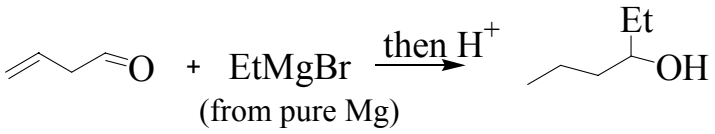
Scheme 6

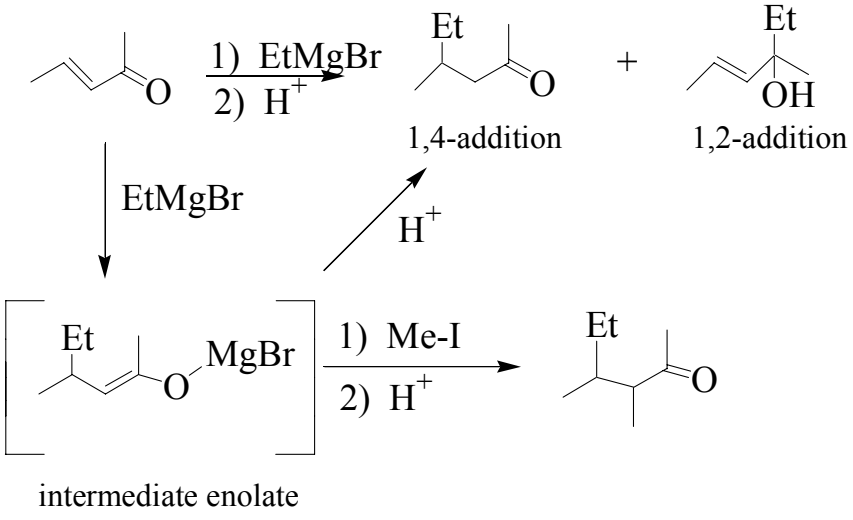


The reactivity of Grignard reagents toward αβ-unsaturated aldehydes and ketones strongly depends on the purity of magnesium used for the generation of the reagents. The high purity magnesium is difficult to obtain and it is expensive. The Grignard reagent prepared from such magnesium reacts with αβ-unsaturated aldehydes and ketones by addition to the carbonyl group preferentially, as illustrated in Scheme 7 by the reaction of ethyl magnesium bromide with crotonaldehyde. On the other hand, regular commercial magnesium reacts, particularly with αβ-unsaturated ketones, in 1,2- and 1,4-addition modes (Scheme 7). The 1,4-

addition is promoted compared with 1,2-addition by cuprous ion Cu^+. The commercial magnesium contains impurity of copper and other transition metals, which catalyzes 1,4-conjugate addition. When a mixture of 1,2- and 1,4-addition products is formed, a deliberate treatment of the Grignard reagent with a cuprous salt, such as CuBr before the reaction with an unsaturated ketone, results in a clean formation of the conjugate adduct only. The 1,4-conjugate addition catalyzed by cuprous ion is of synthetic importance. The mechanism is complex but, almost certainly, it involves the generation of radical intermediates resulting from single electron transfer (as opposed to purely ionic reaction). The intermediate enolate product can be alkylated to introduce an alkyl group α to the carbonyl group (Scheme 7).

Scheme 7





Organolithium reagents[1-6]

This class of reagents encompasses alkyllithium and aryllithium derivatives in which a lithium atom is bonded directly to the carbon atom and lithium amide reagents derived from secondary amines. Typical examples are shown below.

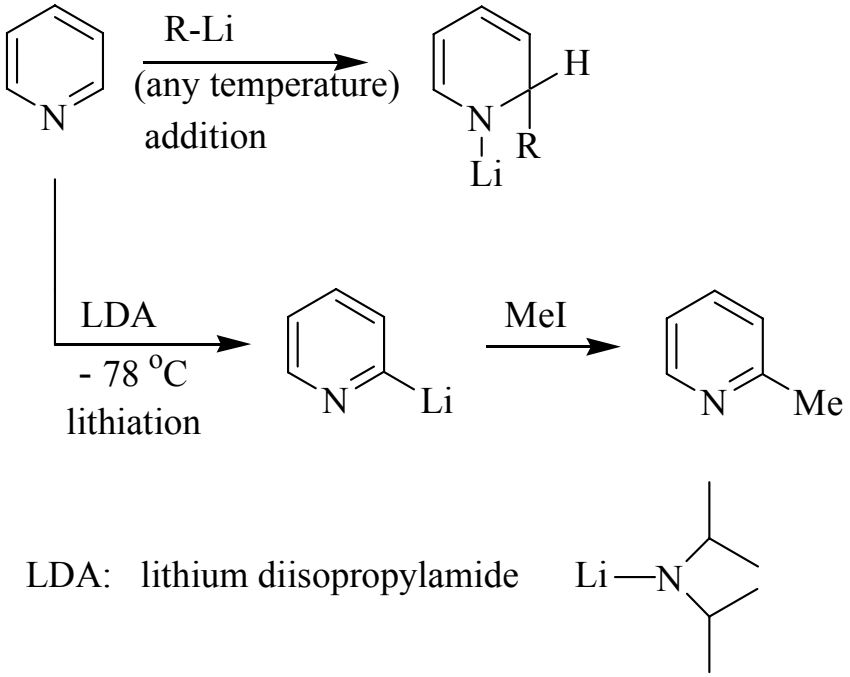
Methyllithium	CH_3-Li	(MeLi)	a strong base and a strong nulceophile
n-Butyllithium	*n*-C_4H_9-Li	(*n*-BuLi)	a strong base and a strong nucleophile
t-Butyllithium	Me_3C-Li	(*t*-BuLi)	a strong base and a strong nucleophile

basicity increases in the following order: MeLi < *n*-BuLi < *t*-BuLi

Phenyllithium	C_6H_5-Li	(PhLi)	a strong base and a strong nucleophile
Lithium diisopropylamide		iPr_2N-Li (LDA)	a strong base and a relatively weak nucleophile

Both types of reagents are strong bases. However, the alkyllithiums and aryllithiums are also strongly nucleophilic (the affinity toward a carbon atom, e.g. in the addition to the carbonyl group), while lithium amide reagents are relatively non-nuclephilic. This remarkable difference is illustrated in Scheme 8 where an alkyllithium or aryllithium reagent undergoes preferentially the addition reaction to the formal C=N bond of pyridine (pyridine is less aromatic than benzene and, as a consequence, has partially localized double bonds), while LDA causes lithiation (hydrogen – lithium exchange) of pyridine.

Scheme 8



Many organolithium compounds, including *n*-BuLi, *t*-BuLi, and LDA, are soluble in hydrocarbons. Important exceptions are MeLi and PhLi, and diethyl ether (ether) or tetrahydrofuran (THF) are the solvents of choice for conducting chemistry with these reagents. The reagents are associated into structurally well-defined clusters in solution. These are complexes in which individual molecules are bonded to each other by a "lithium bond" which is similar in nature to hydrogen bonding. Methyllithium is a solvated tetramer in ether, and *n*-butyllithium is hexameric in hydrocarbons but becomes tetrameric in ether which is a more polar solvent.

Generation of organolithium reagents. *Note: Generation implies that the product is not or cannot be isolated and it is used in situ for the subsequent reaction. Synthesis or preparation means that the product is stable under normal conditions and can be purified and isolated.* The most important methods are based on (i) the reaction of alkyl halides with lithium metal, (ii) lithium – halogen exchange in the reaction of an alkyllithium reagent and an alkyl halide, and (iii) lithium – hydrogen exchange in the reaction of an organolithium reagent with a relatively acidic hydrocarbon portion of a molecule (lithiation). An exchange reaction between an organotin compound and organolithium reagent (transmetallation) is also discussed below.

Organolithium compounds are made industrially by the reaction of organic halides with metallic lithium (i). Reactivity toward lithium is in the order RI > RBr > RCl and fluorides do not react. Typically the reaction is conducted with organic chlorides in ether because lithium chloride, the byproduct, is not soluble in this solvent. The resultant reagent is does not contain lithium chloride.

$$RCl + 2Li \longrightarrow RLi + LiCl \qquad \text{(i)}$$

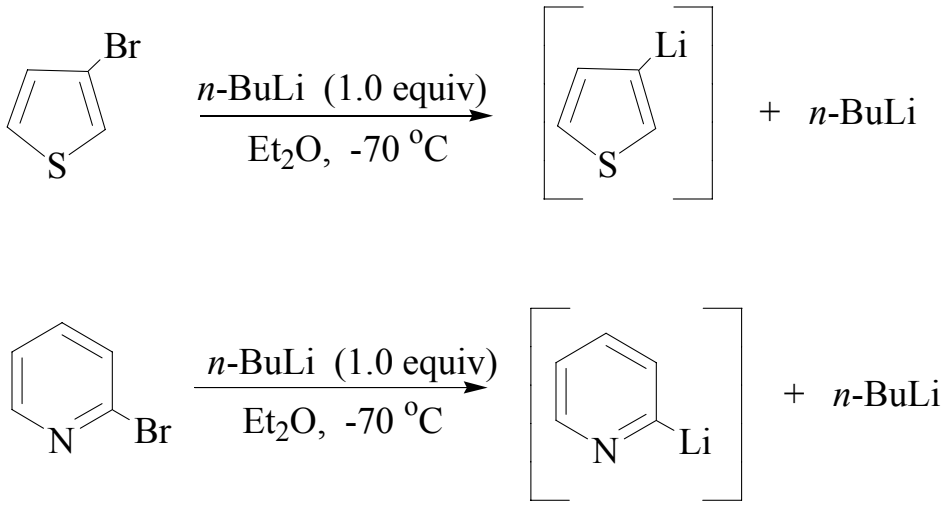
$$RLi + R^1X \rightleftharpoons R^1Li + RX \qquad \text{(ii)}$$

X = I or Br

$$RLi + R^1H \longrightarrow RH + R^1Li \qquad \text{(iii)}$$

In the lithium – halogen exchange reaction (ii) equilibrium lies toward the side giving the organolithium compound whose organic group is better able to accommodate partial carbanionic character (Scheme 9). The method is particularly useful for the preparation of aryl and alkenyl reagents (Csp^2-Li) by reaction of *n*-butyllithium (Csp^3-Li) with aryl and alkenyl halides. With iodo and bromo derivatives the reaction is general, and often proceeds

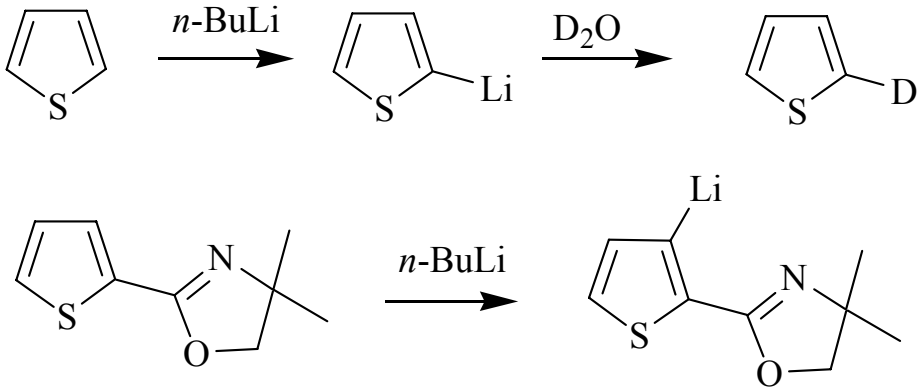
Scheme 9



rapidly (a few minutes) even at very low temperatures (~ -50 ^{o}C). The reaction is normally carried out in ether solvents, though it does take place, albeit slowly, in hydrocarbons. Because lithium – halogen exchange proceeds rapidly under mild conditions, potential side reactions such as alkylation of or elimination from the organic halide are not usually troublesome.

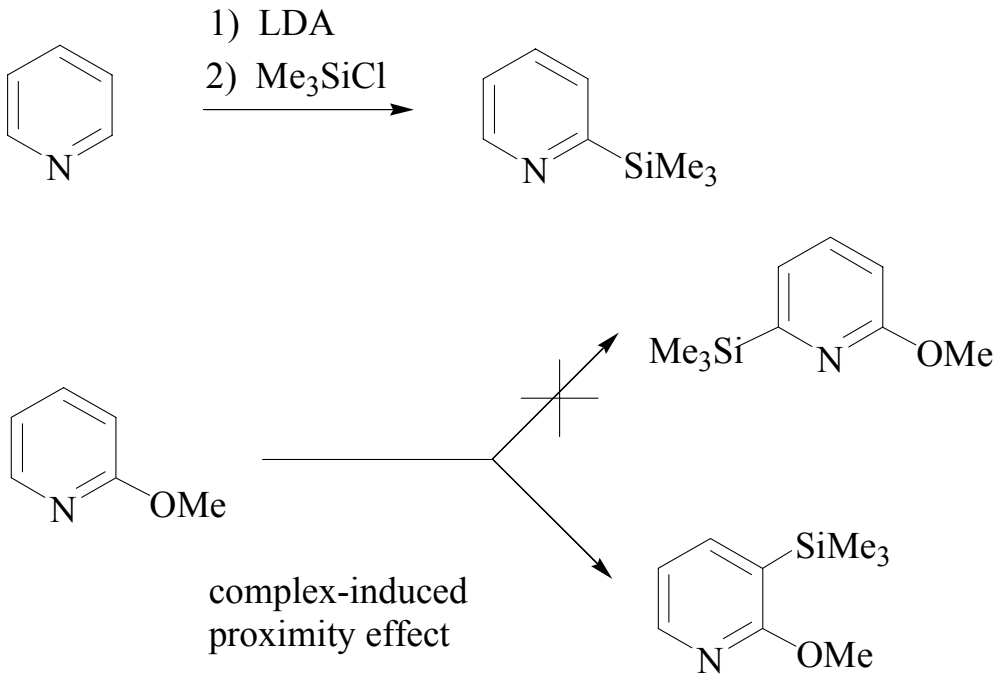
The replacement of hydrogen by lithium in an organic compound (iii above), called lithiation, is perhaps the most versatile method for the generation of organolithium reagents. The simplest lithiations are those of relatively strong hydrocarbon acids ($pK_a \leq \sim 33$) such as 1-alkynes and aryl-substituted methanes. For example, toluene is efficiently lithiated at the methyl group upon treatment of the solution in ether with PhLi at -50 ^{o}C. Heterocyclic compounds including pyridine, thiophene, and furan, in the absence of polar substituents, are lithiated regioselectively at the α position (next to the heteroatom) because the presence of the heteroatom increases acidity of the α-hydrogen atom. An example has already been provided in Scheme 8 above. This type of activation is also induced by some substituents at aromatic and heterocyclic (heteroaromatic and nonaromatic) compounds. Some of these groups also function as protecting groups. The phenomenon is known as **C**omplex-**I**nduced **P**roximity **E**ffect (CIPE) and it is illustrated in Schemes 10 – 13. Selected groups promoting α-lithiation of heterocyclic compounds and ortho-lithiation of aromatic compounds are $-SO_2NR_2$, $-SO_2Ar$, $-CONR_2$, oxazolinyl (Scheme 10), $-CH_2NR_2$, -OR (Scheme 11), $-NR_2$, halogens –F, -Cl, -Br, -I (Scheme 12), and *tert*-butoxycarbonyl (BOC) group (Scheme 13). The *ortho*- and α-promoted lithiation for halogen-substituted substrates is best carried out with LDA to avoid halogen – lithium exchange (see ii above). The directing effect of a substituent can sometimes override the effect of a ring heteroatom (Schemes 10 – 12). CIPE can be explained in terms of complexation of the lithium reagent by the functional group. The lithiation of the position adjacent to the functional group takes place because the reagent in the complex is favorably positioned for the reaction with the adjacent hydrogen atom.

Scheme 10

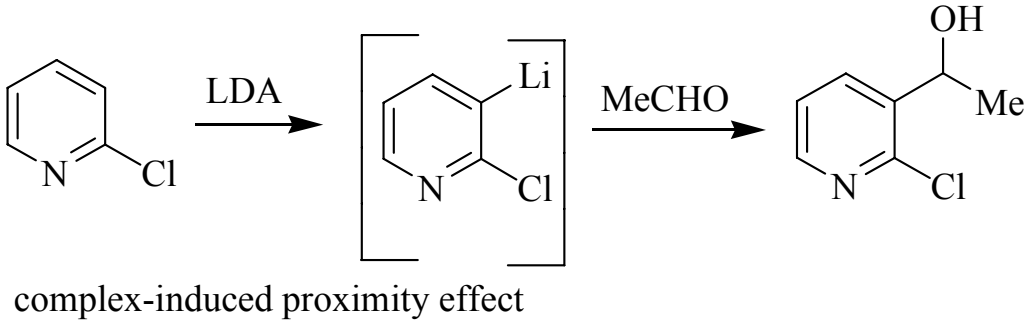


complex-induced proximity effect

Scheme 11

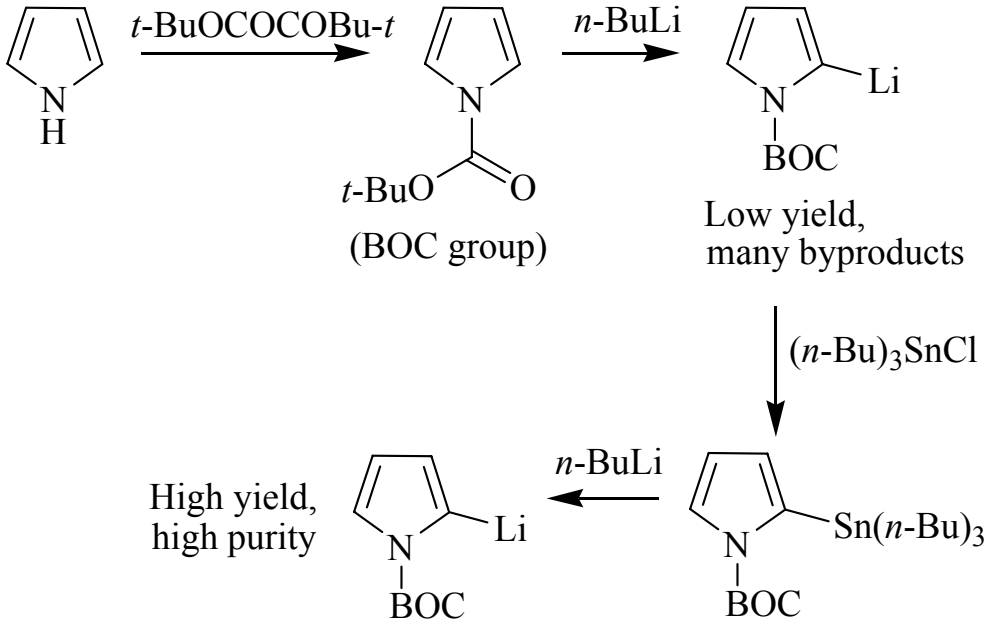


Scheme 12



The tert-butoxycarbonyl (BOC) group is a popular protecting group for secondary amines (Scheme 13). The BOC function is stable toward organometallic bases, such as *n*-butyllithium but is easily removed by treatment with a diluted mineral acid such as hydrochloric acid. As shown in Scheme 13, BOC-protected pyrrolidine undergoes lithiation at the α-position but this process is inefficient and many byproducts are formed. The use of such lithiated compound for a subsequent reaction with an electrophile (for example, ketone or aldehyde) would produce a complicated mixture that would be difficult to separate. A solution to this problem is to react the lithiated BOC-pyrrolidine with tributyltin chloride [(*n*-Bu)$_3$SnCl]. The organotin compounds, including the product of this reaction, are

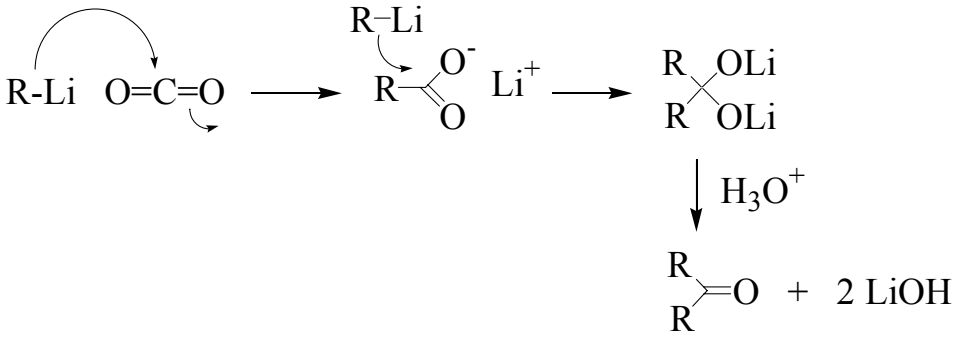
Scheme 13



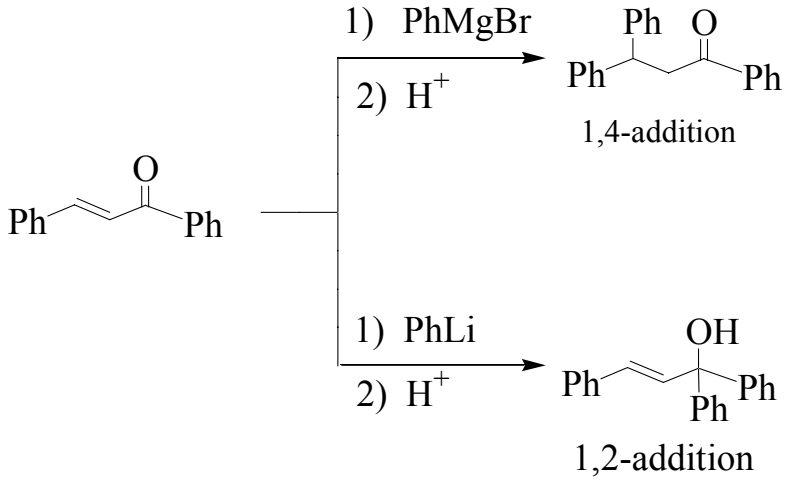
crystalline compounds that are easily purified by simple crystallization. The tin product of this reaction can be easily crystallized in a pure state from the complicated mixture. The subsequent treatment of the pure organotin product with n-butyllithium generates the desired lithiated BOC-pyrrolidine in high yield by tin – lithium exchange reaction. This lithium derivative can be used *in situ* for a subsequent reaction with an electrophile.

Reactivity. In general, organolithium reagents are more reactive than the corresponding Grignard analogs. Several reactions with electrophiles have been illustrated above. Perhaps the most striking difference is the facile synthesis of carboxylic acids by the reaction of Grignards with carbon dioxide (Scheme 5) as opposed to the preparation of ketones by treatment of lithium derivatives with carbon dioxide (Scheme 14). The difference arises from the fact that the lithium compounds are more strongly nucleophilic than Grignard reagents and are able to react with the intermediate resonance – stabilized carboxylate anion. Similarly, carboxylic acids may be converted into ketones.

Scheme 14

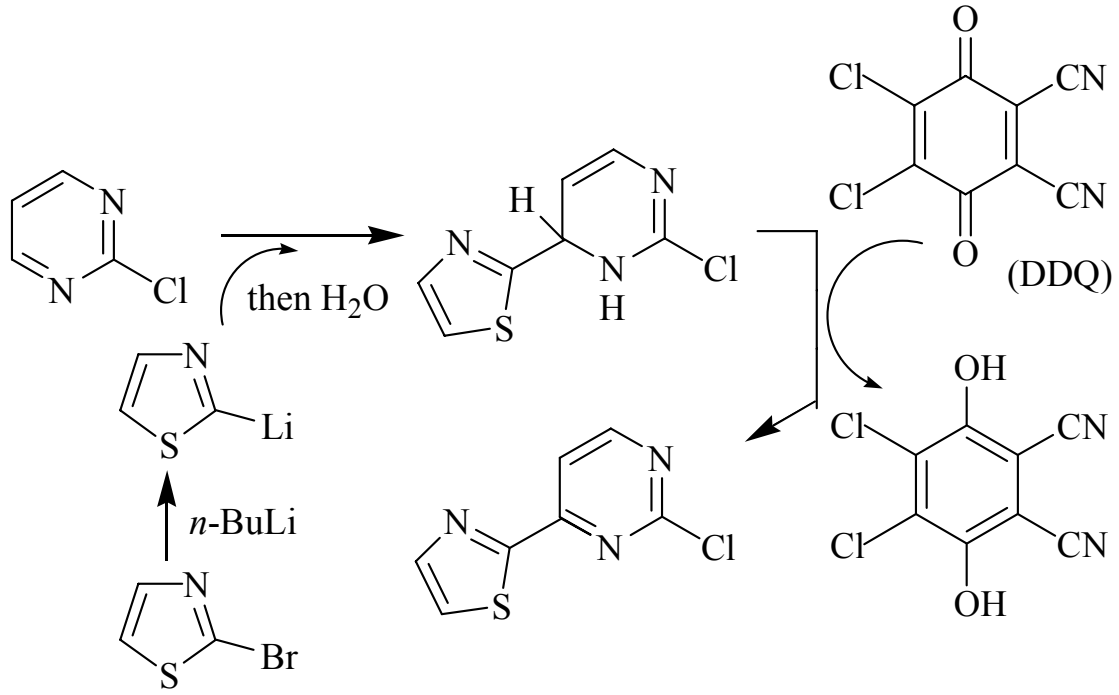


Scheme 15



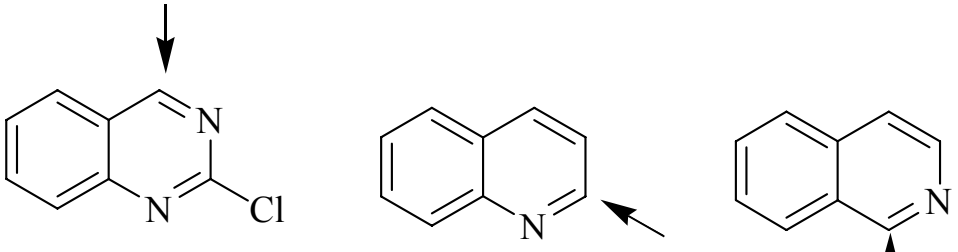
It has been discussed above that Grignard reagents often react with αβ-unsaturated ketones predominantly by conjugate 1,4-addition. This reactivity pattern is catalyzed by impurity of transition metals in commercial magnesium. By contrast, the commercial purification of lithium provides the metal of high purity that is transition-metal free. As a consequence, organolithium reagents undergo predominantly a 1,2-addition to unsaturated ketones, as illustrated in Scheme 15. As with Grignards, the reaction of αβ-unsaturated ketones with lithium compounds in the presence of cuprous ion yields predominantly the 1,4-adduct.

Scheme 16



Organolithium reagents undergo not only the addition reaction to a carbonyl group (C=O) but also to the formal C=N function of pyridazine, pyrimidine, pyrazine, quinazoline, quinoline, and isoquinoline. These addition reactions are of synthetic value. This reaction is particularly useful for 2-chloropyrimidine (Scheme 16) and 2-chloroquinazoline (Scheme 17). As illustrated in Scheme 16, 2-lithiothiazole is generated by the bromine – lithium exchange reaction between 2-bromothiazole and *n*-butyllithium and then allowed to react with 2-chloropyrimidine. The resultant adduct is dehydrogenated (aromatized) by treatment with 2,3-**d**ichloro-5,6-**d**icyano-1,4-benzo**q**uinone (DDQ). DDQ is reduced to a hydroquinone that is easily removed from the organic solution (ether) by extraction with an aqueous solution of sodium hydroxide (hydroquinones are soluble in an aqueous basic solution). It is remarkable that the nucleophilic attack by the organolithium reagent (a hard nucleophile) occurs at a carbon that does not bear a chlorine. The synthetic utility of this synthesis is greatly enhanced by the fact that the chlorine in the final product can efficiently be displaced by the reaction with soft nucleophiles such as alkylthio, arylthio, alkoxy or phenoxy ion, and amines. 2-Chloroquinazoline can be substituted in a similar way. Quinoline and isoquinoline (Scheme 17) can also be reacted with organolithium reagents. The resultant dihydro intermediate products can be aromatized (oxidized) by treatment with nitrobenzene under reflux conditions.

Scheme 17



The addition reaction of organometallic reagents

Organocopper reagents (RCu) and lithium organocuprates (R_2CuLi). The synthetic utility of organolithium compounds is strongly enhanced by their derivatization by the reaction with cuprous salts. Two types of copper(I) reagents are of value in synthesis: organocopper compounds RCu and lithium organocuprates R_2CuLi, where R is alkyl (primary, secondary or tertiary), alkenyl or aryl. They can be prepared from the corresponding lithium reagents by the reaction with copper(I) iodide CuI in ether under an inert atmosphere, as illustrated in Scheme 18 for the reaction with MeLi. The purification of commercial CuI involves continuous extraction with THF in a Soxhlet apparatus, which efficiently removes soluble copper(II) impurities.

Scheme 18

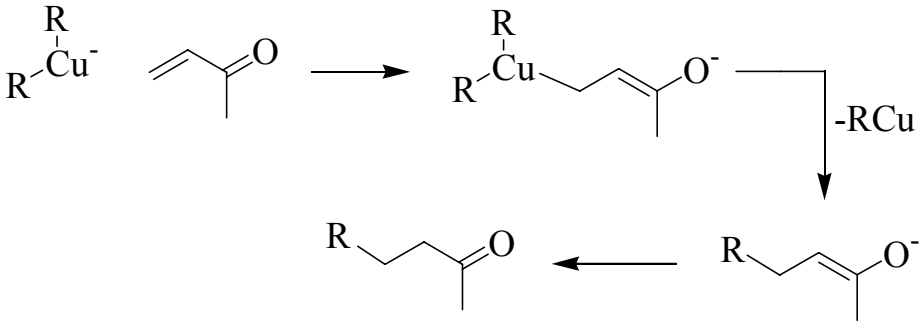
$$\underset{\text{colorless}}{\text{MeLi}} + \text{CuI} \rightarrow \underset{\text{yellow}}{\text{MeCu}} + \text{LiI}$$

$$\text{MeLi} + \text{MeCu} \rightarrow \underset{\text{colorless}}{\text{Me}_2\text{CuLi}}$$

The preparation of these reagents is a joyful experience. Cuprous iodide is weighed in a flask, the flask is capped under an argon or nitrogen atmosphere with a rubber septum, anhydrous ether is added using a syringe, and the mixture is magnetically stirred. During a dropwise addition of methyllithium solution using a syrringe, the unpleasant grey color of CuI is gradually replaced by a beautiful yellow precipitate of methylcopper. After addition of 1 equivalent of MeLi, the continuous titration results in a gradual disappearance of the yellow precipitate and the formation of colorless dimethylcopperlithium reagent. There is no need to know the concentration of MeLi if Me_2CuLi is the desired product because yellow methylcopper is the indicator. The generation of Me_2CuLi is complete when traces of MeCu disappear and a clear colorless solution is formed.

The organocuprates R_2CuLi are more reactive than organocopper compounds RCu. In contrast to lithium reagents RLi, the reagents R_2CuLi are not reactive enough to undergo a 1,2-addition to carbonyl compounds including esters, ketones, and aldehydes. However, a rapid 1,4-addition is observed with αβ-unsaturated aldehydes and ketones. This is illustrated below in Scheme 19.

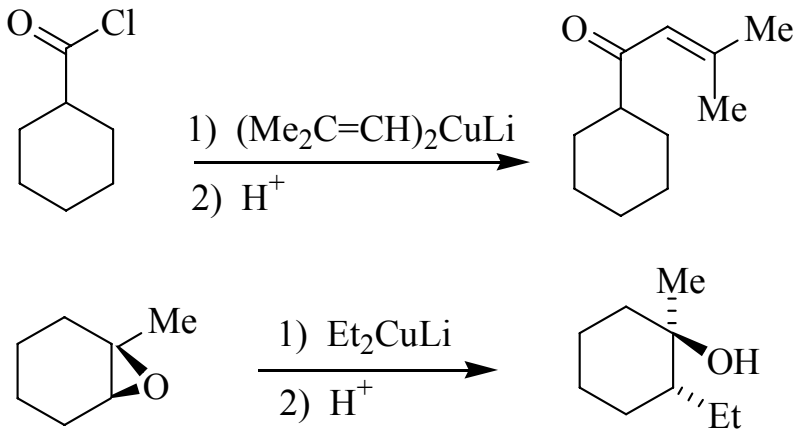
Scheme 19



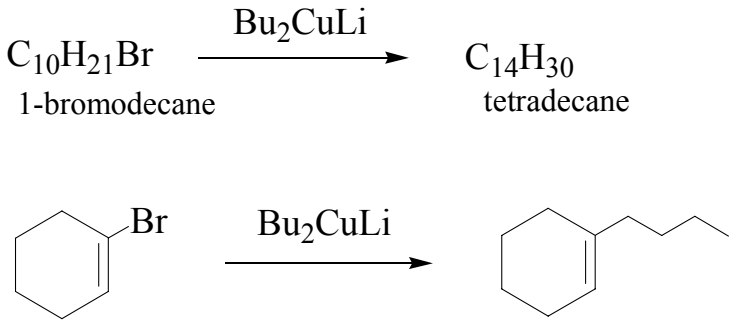
A mechanism apparently involves an intermediate adduct in which copper has a formal oxidation state of +3 and which undergoes a reductive elimination of RCu. Note that the same product can be obtained by conjugate addition reaction of a Grignard reagent RMgX in the presence of a cuprous salt. Both preparations are highly efficient and the choice of either Grignard or lithium reagent is normally dictated by the availability.

Efficient synthesis of ketones involves the reaction of organocuprates with acid chlorides (Scheme 20). Alcohols are formed by opening of epoxides. The reaction proceeds with inversion of configuration at the less substituted carbon atom of the epoxide, as in the S_N2 process (Scheme 20). Organocuprates also undergo reaction with alkyl (primary or secondary), alkenyl, and aryl bromides and iodides (but not chlorides), by displacement of halide ion in a process known as a coupling reaction (Scheme 21). Yields are generally high.

Scheme 20



Scheme 21



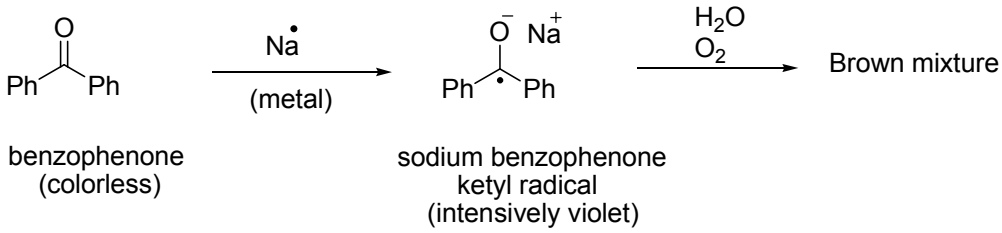
Experimental set-up. At room temperature there is a lot of water adsorbed on the surface of glass. Organolithium reagents are water sensitive. On contact with water they decompose rapidly generating the parent organic compounds and lithium hydroxide. Accordingly, the glassware for conducting organometallic synthesis must be absolutely dry. Organolithium reagents also react with molecular oxygen to generate organic lithium peroxides which can decompose to organic lithium oxides in a subsequent reaction with the starting organolithium reagent. If the precautions to remove traces of water and oxygen are not taken, the overall effect is a greatly reduced efficiency of the desired reaction at the expense of the unwanted reactions with water and oxygen.

The experiments with organolithium reagents must be conducted in a rigorously dry glassware under an atmosphere of an inert gas such as dry nitrogen. The preferred method of drying of glassware is simply storing the glassware in an oven at 110 – 140 ^{o}C. The glassware should be assembled hot and cooled in a stream of dry nitrogen by simply inserting a tubing with nitrogen flowing through it. The flow of nitrogen should be at least 25 ml/min. Alternatively, the cold glassware is assembled at room temperature and the set-up is flushed constantly with dry nitrogen as described above during an external heating using a high-temperature heat gun capable of heating the air to at least 200 ^{o}C. The application of either procedure will give a dry glassware that is also filled with an inert gas. With the tubing still inserted in the assembly and nitrogen flowing through the system, the openings are capped with rubber septa. Now, the only uncapped opening bears the nitrogen tubing. One of the rubber septa on the set-up is then connected to a nitrogen line through a surgical needle of about 1 mm of inside diameter. Finally, the nitrogen tubing is slowly removed from the system and the opening is capped with a rubber septum. The resulting assembly is dry, under an atmosphere of inert gas, and connected to a nitrogen line through a metal needle only. The nitrogen line is vented to the atmosphere through a bubbler, so that the atmospheric pressure is maintained inside the assembly regardless of changes in temperature or volume following the addition of reagents to the flask. Only liquid materials can be introduced to the flask through the rubber septa by using syringes equipped with a metal needle. Any solid material must be dissolved in a suitable solvent first. The rubber septa, syringes, and needles are dried by storing in the presence of P_2O_5 in a desiccator.

Solvents. Diethyl ether (ether) and tetrahydrofuran (THF) are the most common solvents for conducting organolithium chemistry. Hydrocarbon solvents are used sparingly because of their limited ability to dissolve most organic substrates. The hydrocarbon solvents also decrease reactivity of organolithium reagents by allowing the formation of less reactive organolithium aggregates. The preferred way to dry ether and THF is by using benzophenone and metallic sodium under an atmosphere of dry nitrogen. Specifically, the solvent is placed in a two-neck flask, normally a two-liter flask, equipped with a distillation column and a receiver. The receiving flask is usually a one-liter flask with a rubber septum–closed neck for an easily withdrawal of the distilled solvent using a large, normally 50-ml or 100-ml syringe. The entire assembly is placed in a heating mantle sitting on a large magnetic stirrer. A stirring bar is introduced to the flask trough the remaining neck, and the system is flushed with dry nitrogen using an atmospheric pressure-equalized nitrogen line. Then the nitrogen flow is greatly reduced to help introduce the solvent to the flask through the open neck without any spilling accident. Note that the entire distillation still is under the atmosphere of nitrogen. The flask is not heated at this time. The flask with the solvent is stirred and charged

with benzophenone (about 10 g for one liter of solvent) followed by incremental addition of small pieces of metallic sodium (about 1.3 g), and then the open neck is closed with a Teflon stopper. The chemistry is shown in Scheme 22. A single electron is transferred from metallic sodium to benzophenone to generate sodium benzophenone ketyl that is intensively violet in color. This is a visually beautiful reaction that is followed by formation of a visually unpleasant brown material as the ketyl is decomposed in the reactions with water and oxygen present in the solvent. The violet color persists after the solution becomes anhydrous and oxygen-free, which means that the solvent is ready to be distilled. Normally, the still is kept under a gentle reflux under a nitrogen atmosphere all the time, the temperature is increased for distillation only, and it may be necessary to charge the solvent with an additional amount of benzophenone and/or metallic sodium when the contents loses the violet color. The system benzophenone/sodium also reduces dangerous peroxides that may be present in an ether solvent that had been exposed to oxygen and sunlight. Anyway, it is always a good practice to test the ether solvent for the presence of peroxides by using paper strips covered with potassium iodide and starch. The test is very simple and consists of immersing the paper strip in the solvent. The peroxides oxidize iodide ion to molecular iodine which forms a blue complex with starch. Conversely, the absence of the blue color indicates the absence of peroxides in the solvent and the conclusion is that the solvent is safe to use.

Scheme 22



A classical way to dry ether or THF is to store these solvents over metallic sodium. Normally, sodium is pressed into a wire on a special press to increase the surface area of the contact with the solvent. This method is not recommended because the drying process is slow, as it takes several days for a complete removal of water, and there is a danger of the formation of peroxides if the air is not completely removed. The peroxide test mentioned above must always be conducted with ether and THF dried with metallic sodium.

Finally, a totally unacceptable method for drying of ethers with a simultaneous removal of peroxides is with the use of lithium aluminum hydride ($LiAlH_4$). This powerful reducing agent reacts efficiently with traces of water and efficiently reduces dangerous peroxides. Unfortunately, it can also be dangerously explosive when heated to the temperature as low as 100 ^oC in the solid state. An accident may happen easily when a mixture of $LiAlH_4$ and ether or THF is left unattended resulting in the complete distillation of the solvent thus leaving the explosive solid residue.

Note on the use of glass-on-glass connections. The glass-on-glass internal surfaces may "freeze" relatively quickly, that is, may become inseparable under basic conditions. More specifically, an organolithium reagent, a mixture after quenching the organometallic reagent with water, sodium metal, and amines may provide such disastrous basic conditions when

accidentally placed between two glass surfaces. A good example is an attempted storage of an organolithium reagent, such as solution of *n*-butyllithium in hexanes, in a glass flask closed with a glass stopper, only to find out after several hours later that the stopper cannot be removed from the flask. In this incident, shaking of the flask caused migration of the solution between the neck of the flask and the stopper, where *n*-butyllithium underwent decomposition on contact with atmospheric moisture producing lithium hydroxide. The addition of metallic sodium to the distillation still described above may also provide conditions for such an unwanted glass-with-glass reaction if the glass stopper is used. Finally, a common mistake is to store a liquid amine in a glass flask closed with a glass stopper. A solution to the problem is the use of a stopper made of a different material or a glass stopper the surface of which is covered with an inert material. Unfortunately, the common rubber stoppers are not elegant, the inert Teflon stoppers are not common in the laboratory use, and the application of a silicon grease to modify the glass joints is unpleasantly messy. The practical solution is to cover the glass stopper with a Teflon tape. The proper procedure calls for immobilizing the end of the tape on the stopper with one hand and wrapping the stopper using the second hand, slightly pulling the tape so that it undergoes elongation and firmly covers the stopper. Finally, the Teflon-covered stopper is placed in a neck of a glass flask, and firmly twisted in the direction of the tape application to form an air-tight connection. Once modified, such stoppers can be used many times and provide tight connections even in highly demanding reduced-pressure applications.

Handling of organolithium reagents. The commercial reagents, such as a solution of methyllithium in ether or diethoxymethane, a solution of *n*-butyllithium in cyclohexane or hexanes (a mixture of hexane isomers), and a solution of phenyllithium in di-*n*-butyl ether are supplied in dark bottles, usually 100 ml, that are capped with a SureSeal^{TM} metallic capsule over a neoprene gasket (a chemically inert rubber). The capsule has an opening in the middle so that the solution can be accessed through the gasket by using a syringe equipped with a metallic needle. The needle should be long enough to extend to the liquid in the bottle. Note that an attempted removal even of a small volume of the solution would be resisted by a buildup of a reduced pressure inside the bottle. A forced removal would be possible with the air entering the bottle through the hole around the needle. Once in the bottle, the air would degrade the reagent producing a lithium alkoxide and lithium hydroxide by the reaction with molecular oxygen and water, respectively. Therefore, it is imperative that any volume of the reagent removed from the bottle is replaced by the equal volume of dry nitrogen. Experimentally, it is done by inserting a needle from a nitrogen line into the neoprene gasket, making sure that the tip of the needle is above the surface of the liquid, followed by removal of the reagent solution by using a syringe equipped with a long needle. For a prolonged storage the bottle must be additionally closed with a plastic screw-cap that tightly closes any openings in the gasket.

Titration of organolithium reagents. Commercial alkyllithium and phenyllithium reagents are accompanied with a lot analysis, but often freshly obtained solutions have obviously deteriorated, being dark colored and cloudy, and the solutions deteriorate substantially on storage as visualized by the appearance of sediment. Thus, accurate determination of the concentration of the reagents is generally necessary. Several methods of titration have been described.[1] Some of them use the formation of a colorless monoanion

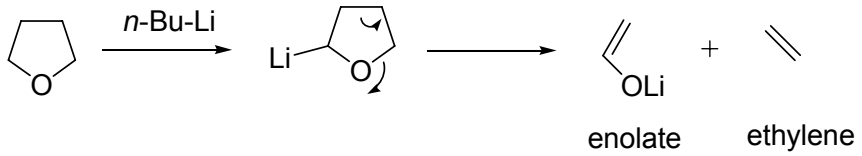
followed by the appearance of a color at the onset of the generation of a dianion. Diphenylacetic acid is a popular reagent for the titration of alkyllithium solutions.[3] It has the advantage of being solid, stable on storage, and easily weighed. In a typical titration experiment (Scheme 23), a sample of diphenylacetic acid (typically 0.25 g) is weighed into a 25-ml Erlenmeyer flask that is then flushed gently with nitrogen and capped with a rubber septum. Anhydrous THF (5 ml) is added to the flask using a syringe. The alkyllithium solution is run from a syringe to the flask until the addition of the next drop results in the appearance of the onset of the yellow color. The yellow color indicates the beginning of the formation of lithium α-lithiodiphenylacetate after complete ionization of the carboxyl group.

Scheme 23

$$(C_6H_5)_2CHCOOH \rightarrow \underset{\text{(colorless)}}{(C_6H_5)_2COO^-Li^+} \rightarrow \underset{\text{(yellow)}}{(C_6H_5)_2CLiCOO^-Li^+}$$

Generation of organolithium reagents.[1,2] The generation *in situ* of organolithium reagents containing the C(sp^2)-Li or C(sp)-Li moieties can conveniently be achieved by direct lithiation (hydrogen – lithium exchange) of the corresponding C-H substrates or bromine – lithium exchange with the corresponding C-Br substrates by the reaction with commercially available *n*-butyllithium under an inert atmosphere. Typically, these reactions are completed in less than one hour even at -50 °C in ether or THF. It should be warned that any reaction of *n*-BuLi in THF should not be conducted above 0 °C. Lithiation of THF takes place at higher temperatures and the resultant intermediate α-lithiotetrahydrofuran undergoes decomposition to lithium enolate of acetaldehyde and ethylene as indicated in Scheme 24.

Scheme 24



Synthesis of 2-chloro-4-(2-thienyl)pyrimidine[4] (3)

Solvent: Anhydrous ether or THF

Thiophene: Density 1.05 g/ml. This liquid substrate should be dried with freshly activated (250 °C, 1 mmHg) molecular sieves 3A or 4A. A bottle with thiophene (typically 25 – 100 ml) should be charged with several pellets of the sieves. Water is removed after several hours.

***n*-Butyllithium:** A commercial solution should be titrated to determine concentration of the reagent. The use of a fresh bottle without titration may result in a lower yield of the final product **3** (Scheme 25).

2-Chloropyrimidine: Pure compound is in the form of white crystals, mp 63 – 64 °C. The commercial product is often contaminated with 2-hydroxypyrimidine and is brown. For

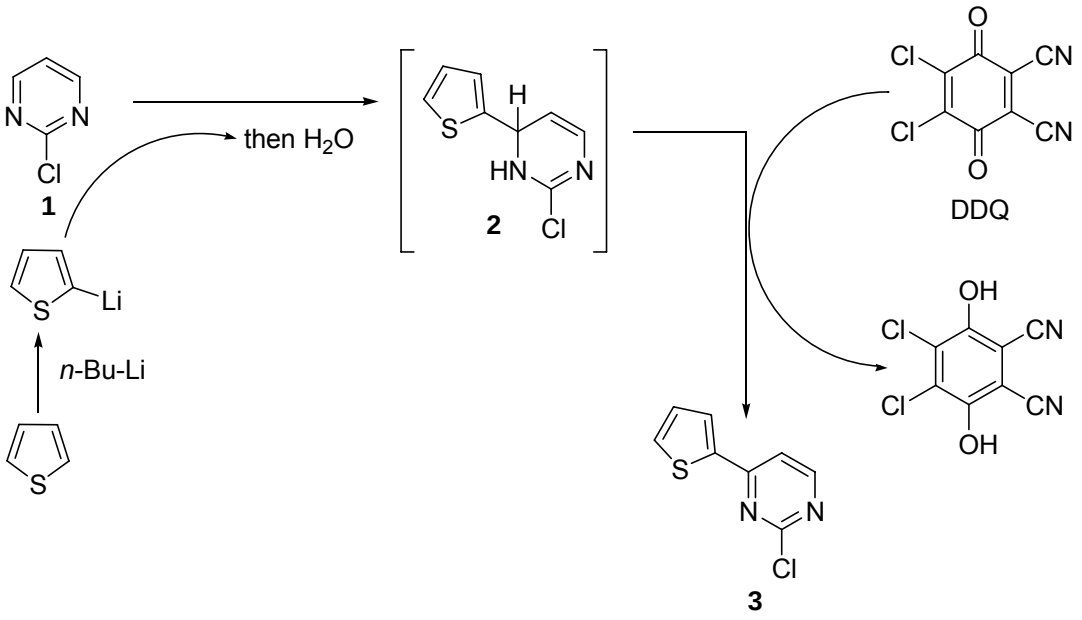
purification by crystallization the commercial product is dissolved in a small amount of dichloromethane (bp 40 °C) and the warm solution is filtered from insoluble material, gently diluted with a 4-fold volume of warm hexanes, and cooled in the refrigerator.

2,3-Dichloro-4,5-dicyanobenzoquinone (DDQ): Commercial material (Aldrich) can be used without purification.

TLC plates (silica gel), **NaOH** (3M), **Na_2SO_4** (anhydrous drying agent).

Techniques: Handling of air-sensitive reagents, chromatography, crystallization, mp, ^{1}H NMR spectroscopy, GC-MS

Scheme 25



All additions of liquid materials in the following procedure are done using syringes. Weights or volumes of the reagents should be calculated from the given amounts in mmols. A round bottom 50-ml flask equipped with a stirring bar and a rubber septum is charged under a nitrogen atmosphere with ether or THF (10 ml) and thiophene (10 mmol). The solution is cooled to 0 °C on an ice bath, stirred, and treated dropwise with a solution of *n*-butyllithium (5 mmol) for 15 min. Thus generated solution of 2-thienyllithium is cooled to -30 °C on a dry ice/acetone bath and treated dropwise at -30 °C with a solution of 2-chloropyrimidine (**1**, 4.5 mmol) in ether or THF (10 ml) (the solution is prepared in a separate flask under a nitrogen atmosphere). The mixture is stirred at -30 °C for 30 min and then the temperature is allowed to rise to 0 °C during subsequent 30 min, within which time the progress of the reaction is monitored by thin layer chromatography (TLC) on silica gel eluting with hexanes/dichloromethane (1:1). As soon as substrate **1** is consumed, the mixture is quenched with water (7 mmol) in THF (1 ml), stirred, and treated with a solution of DDQ (5 mmol) in THF (5 ml) to effect aromatization of intermediate dihydropyrimidine **2**. The mixture is stirred at 25 °C for 10 min, cooled to 0 °C, treated with hexanes (10 ml) and then with a cold aqueous solution of sodium hydroxide (12 mmol), and stirred at 0 °C for 5 min to remove a hydroquinone resulting from reduction of DDQ (a disodium salt of the hydroquinone remains in the aqueous layer). The top organic layer is removed using a pipette equipped with a rubber

bulb and the remaining aqueous residue is extracted again with THF/hexanes or ether/hexanes (1:1, 25 ml). The combined organic extracts are dried with anhydrous sodium sulfate and then concentrated on a rotary evaporator to give a crystalline residue of product **3**. Analytically pure product **3** is obtained by crystallization from dichloromethane/hexanes or toluene/hexanes, mp 128 – 129 ^{o}C, yield 79%.[4]

Post-synthetic assignments

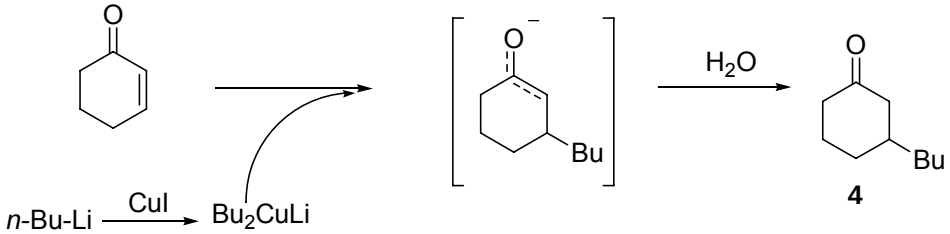
1. Record mp and calculate yield of **3**.
2. Record in $CDCl_3$ solution and analyze the ^{1}H NMR spectrum of **3**. Assign all proton absorptions and calculate coupling constants.
3. Use a GC-MS instrument (electron impact ionization, EI) to analyze purity of **3**. The product contains one chlorine atom and one sulfur atom in the molecule, which should be reflected in the intensity of the M^++2 peak relative to the molecular ion peak M^+. Calculate the theoretical value (M^++2)/M^+ and compare it to that of the experimental spectrum.
4. The chlorine atom in product **3** can easily be displaced by nucleophiles. What is the reason for keeping the solution cold after addition of sodium hydroxide during workup? Also, what can happen if the extraction of the basic solution is not done quickly?

Synthesis of 3-butylcyclohexanone[5] (**4,** Scheme 26)

Solvent: Anhydrous THF.
n-Butyllithium: See the comment above.
2-Cyclohexen-1-one: Density 0.99 g/ml, bp 171 – 173 ^{o}C. The commercial reagent may be dried with freshly activated molecular sieves 3A or 4A.
Cuprous iodide (CuI): The commercially available salt should be continuously extracted with THF in a Soxhlet extractor for 12 h and dried under a reduced pressure at 25 ^{o}C. This procedure removes brown impurity and cupric (Cu^{2+}) salts. The cuprous iodide thus purified remains pure under a nitrogen atmosphere for several months. This purification is not necessary if a commercial salt of high purity is purchased.
Methanol (CH_3OH), **ammonium chloride (**NH_4Cl, saturated solution in water), **sodium thiosulfate** (2% $Na_2S_2O_3$ in water), **magnesium sulfate or sodium sulfate** ($MgSO_4$ or Na_2SO_4, anhydrous drying agent).

Techniques: Handling of air-sensitive reagents, distillation, ^{1}H NMR spectroscopy, GC-MS

Scheme 26



To a suspension of cuprous ioidide (0.89 g, 4.65 mmol) in anhydrous THF (12 ml) under a nitrogen atmosphere at -50 ^{o}C was added a solution of n-butyllithium (9 mmol). The dark mixture was cooled to -70 ^{o}C and treated with 2-cyclohexenone (0.29 g, 0.3 ml, 3 mmol)

in anhydrous THF (3 ml). The mixture was stirred for 30 min, during which time the temperature was allowed to reach -20 ^{o}C, quenched with methanol (3 ml), warmed to room temperature, and then poured into saturated aqueous ammonium chloride (20 ml). After addition of ether (15 ml) the mixture was stirred for 30 min at which time the layers were separated and the aqueous phase was extracted once with ether. The combined organic layers were washed with a 2% solution of sodium thiosulfate and dried over magnesium sulfate. Concentration on a rotary evaporator followed by Kugelrohr distillation (40 mmHg) gave an analytically pure product **4**.

Post-synthetic assignments

1. What is the role of sodium thiosulfate in the work-up?
2. Estimate the temperature for the Kugelrohr distillation of **4** at the suggested pressure of 40 mmHg.
3. Determine the yield of **4**.
4. Record and analyze IR, ^{1}H NMR, and mass spectra of **4**.
5. The conjugate addition reaction (Scheme 5) generates an intermediate enolate anion that can be methylated by treatment with methyl iodide.[5,6] This reaction produces two isomeric products. Suggest their structures. Which is the major isomer and why?

Organolithium reagents: references

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CYANINE DYES

Cyanine dyes (Figure 1) are π-conjugated molecules containing two terminal heterocyclic subunits and the central polymethine linker.[1-3] Such conjugated molecules show absorption and fluorescence that are a function of the structure of the three moieties. The end subunits containing a nitrogen atom may be identical or different. The first member of cyanine compounds was synthesized in 1856. Several cyanine dyes are natural compounds. By changing the length and substitution of the polymethine bridge and/or the structures of the terminal moieties, molecules can be designed with absorption and fluorescence ranging from the blue visible region (> 400 nm) to the near-infrared (NIR) region (> 700 nm) of the electromagnetic spectrum. Their common names denote the number of methine (CH=) groups in the polyene chain. For example, the general structures in Figure 1 with n = 0 and n = 3 are referred to as monomethine and heptamethine cyanines, respectively. Another trivial nomenclature is based on the number of vinylene (CH=CH) moieties in the polyene bridge. Thus, trimethine cyanines (n = 3 in Figure 1) are carbocyanines, pentamethine cyanines are dicarbocyanines, and heptamethine cyanines are tricarbocyanines.

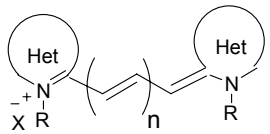


Figure 1. General structure of cyanine dyes

The systematic nomenclature adopted by Chemical Abstracts is substantially different from the IUPAC recommendations. By changing the length and substitution of the polymethine bridge and/or the structures of the terminal moieties, molecules can be designed with absorption and fluorescence ranging from the blue visible region (> 400 nm) to the near-infrared (NIR) region (> 700 nm) of the electromagnetic spectrum. The monomethine and trimethine cyanines generally show absorption in the visible region, and each extension of the chromophore by one vinylene moiety (CH=CH) causes a batochromic shift of about 100 nm. Depending on substituents, absorption of pentamethine derivatives can reach a near-infrared (NIR) region (> 700 nm), and heptamethine cyanines may show absorption beyond 1000 nm. Cyanine dyes have narrow absorption bands and extremely high extinction coefficients, often exceeding 200,000 $M^{-1}cm^{-1}$. Cyanine dyes are mildly fluorescent in solution. The fluorescence efficiency is greatly increased upon binding of the dyes with nucleic acids or proteins as a result of the rigidization of the fluorophore. NIR cyanine dyes are an important class of compounds that have found numerous applications including biosensors and biolabels. Since the NIR region (> 700 nm) is inherently a region of low interference (Figure 2), it is well suited for analytical techniques using high complexity samples. Very low detection limits can be achieved using NIR fluorescence because of high molar absorptivity and substantial quantum yield of fluorescence of NIR dyes as well as the virtually non-existent background interference and high efficiency of semiconductor laser and detection systems.

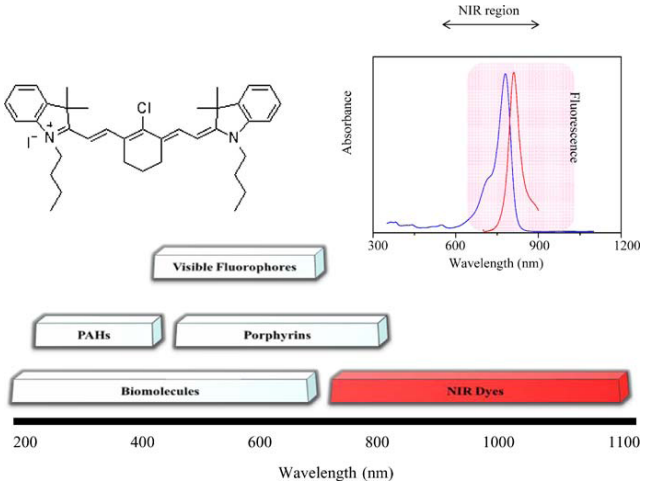
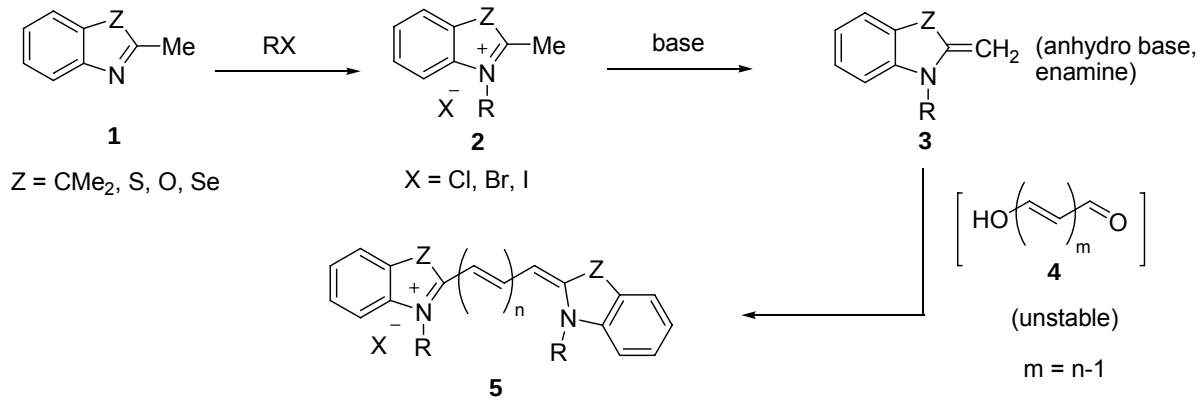


Figure 2. Absorption and fluorescence of near infrared dyes.

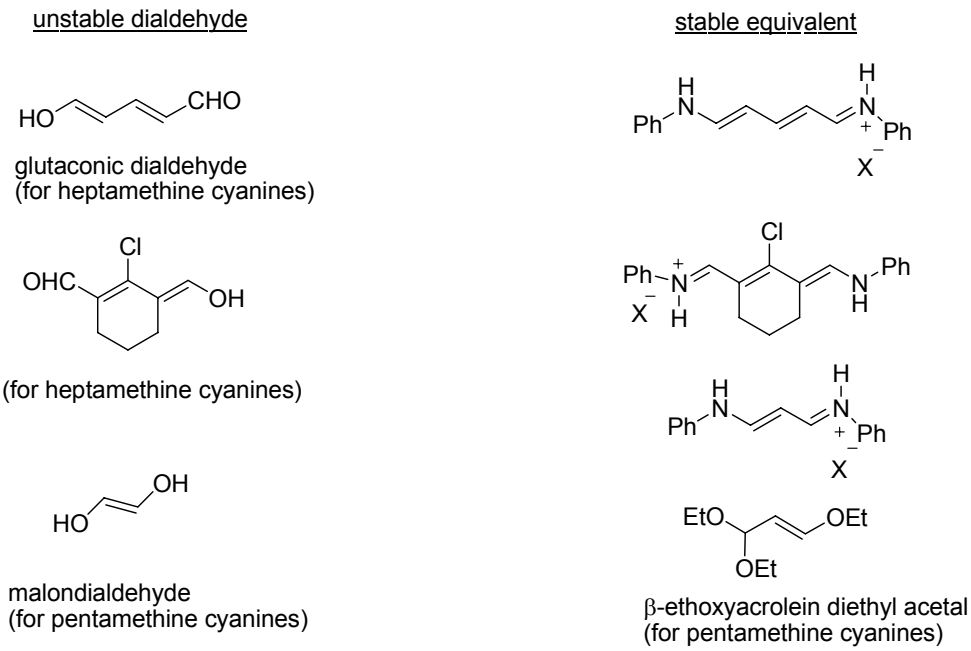
The optimal wavelength range for *in vivo* fluorescence excitation and emission is determined by tissue optical properties.[4] For example, hemoglobin has strong absorption at wavelengths less than about 600 nm and there can be significant background fluorescence from endogeneous biomolecules up to about 680 nm. There is a large increase in light penetration and a large decrease in background fluorescence as wavelengths increase from about 600 nm to 780nm.[4] The NIR luminescence techniques reported recently are overwhelmingly bioanalytical or biologically related analyses that take advantage of the low interference of the NIR spectral region. Fluorescence *in vivo* imaging has become one of the major foci of interest. Review articles and research reports summarize several cancer detection applications using NIR fluorescence.[4-13] Other reviews and original research papers describe fluorescent metal ion sensors,[14-20] binding with nucleic acids,[21-24] and interaction with proteins,[24-35] among others.[36—51]

Synthesis of pentamethine and heptamethine cyanine dyes is illustrated in Scheme 1. In the first step, a heterocyclic substrate **1** is alkylated at the nitrogen atom, which activates the adjacent methyl group for proton removal in the resultant iminium cation **2**. This deprotonation can be accomplished by treatment of **2** with such a week base as pyridine or sodium acetate. The intermediate product **3** is an enamine that is often called anhydrobase. Compound **3** is a direct precursor to the cyanine dye **5** by the reaction with a dialdehyde **4** or a synthetic equivalent such an iminium salt derived from **4** or an analog. The problem is an instability of conjugated dialdehydes. By contrast, the corresponding iminium salts are stable solid compounds that can easily be purified by crystallization, and their reaction with anhydrobases yield the same cyanine products. Several dialdehydes and their stable iminium equivalents are shown at the bottom of Scheme 1. Their synthesis normally involves the generation of the unstable dialdehydes that are not isolated but immediately allowed to react with an amine, typically aniline, under acidic conditions. Note that the dialdehydes are shown with one aldehyde group in an enol form which results in an extended conjugation of the molecule. The imine derivatives are shown in a similar way. Acetals are another class of stable equivalents of dialdehydes.

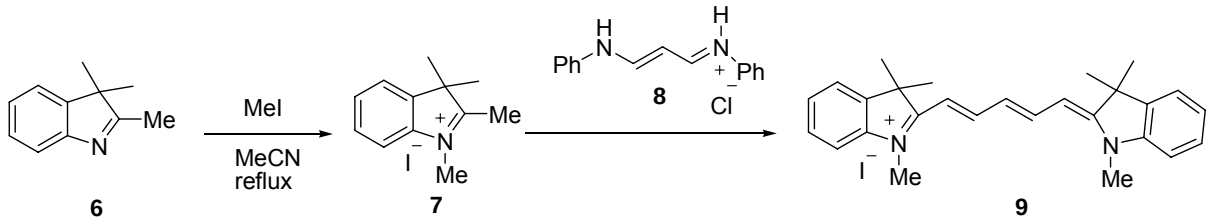
Scheme 1



Stable equivalents of dialdehydes **4**: and analogs



Scheme 2



Synthesis of indolium pentamethine cyanine 9 (Scheme 2). This compound absorbs in the visible region of the electromagnetic spectrum (λ_{max} 637 nm in methanol).

2,3,3-Trimethylindolenine or 2,3,3-trimethyl-3*H*-indole (6): Density 0.99 g/ml, bp 228-229 ^{o}C
Iodomethane or methyl iodide: Density 2.28 g/ml, bp 41-43 ^{o}C
Malonaldehyde bis(phenylimine) hydrochloride (8): Mp 218 ^{o}C (dec)
Anhydrous acetonitrile (CH_3CN): Solvent
Acetic anhydride $(CH_3CO)_2O$: Solvent
Sodium Acetate (CH_3COONa): Base
Methanol and ether: Solvents for crystallization

Techniques: Chromatography, crystallization, ^{1}H NMR, ^{13}C NMR

1,2,3,3-Tetramethyl-3*H*-indolium iodide (7). A solution of indolenine **6** (0.48 g, 0.49 ml, 3 mmol) and methyl iodide (2.13 g, 0.93 ml, 15 mmol) in anhydrous acetonitrile (30 ml) was maintained under a nitrogen atmosphere and heated under reflux for 12 hours. After cooling the solution was concentrated to half the volume on a rotary evaporator and slowly diluted with ether (50 ml). The resultant crystalline product **7** was collected by filtration and dried in a desiccator under a reduced pressure; yield 0.XX g (XX%), mp 258 ^{o}C (dec).

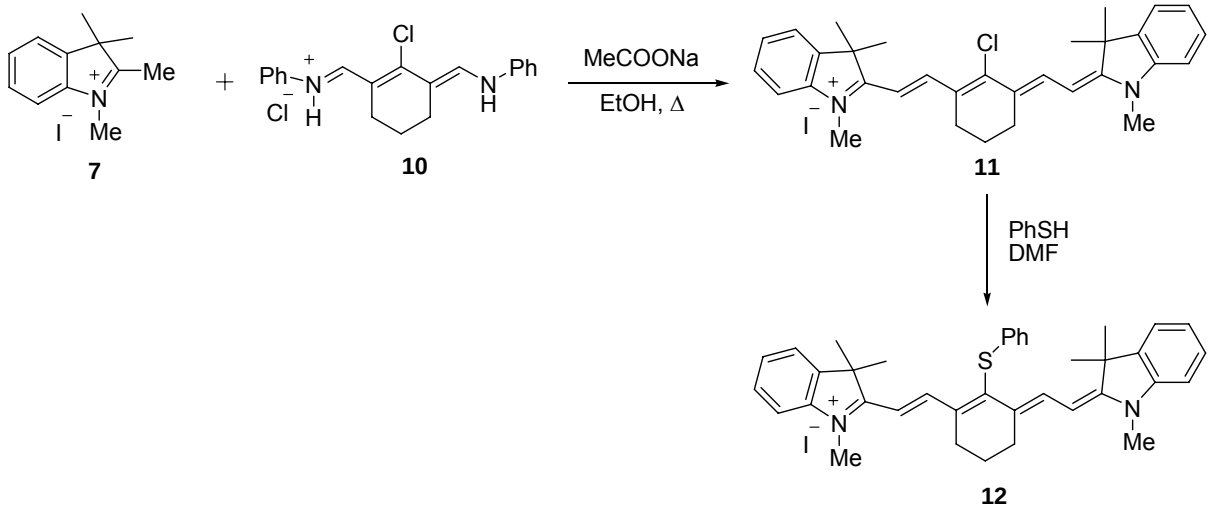
Cyanine 9. A mixture of salt **7** (500 mg, 1.66 mmol), bis-iminium salt **8** (215 mg, 0.83 mmol) and NaOAc (139 mg, 1.66 mmol) was heated in acetic anhydride (10 mL) at 90 ^{0}C for 2 hours under a nitrogen atmosphere, then cooled and filtered. The filtrate was concentrated under reduced pressure on a rotary evaporator. For crystallization, the residue was dissolved in a minimum amount of warm methanol and the solution was gently diluted with ether (caution, bp 35 ^{o}C only!) until the mixture became cloudy. Cooling in the refrigerator for several hours gave 500 mg (85%) of **9** as a green solid; mp > 250 ^{0}C; λ_{max} 637 nm (in methanol).

Post-synthetic assignments

1. Analyze purity of the product by TLC. A mixture of methanol and ether or chloroform is the suggested eluent because this cyanine is highly polar.
2. Record and analyze ^{1}H NMR and ^{13}C NMR spectra of the product for a solution in DMSO-d_6.
3. Record electron absorption spectrum of the cyanine for a solution in methanol and compare λ_{max} with the provided value.
4. Record ^{1}H NMR spectrum of the substrate **8** in DMSO-d_6 solution and determine which tautomer is present (fully conjugated amino-imine vs. bis-imine).
5. Name cyanine **9** according to the IUPAC approach. Consult references[52-57] for names of similar dyes.

Synthesis of indolium heptamethine cyanines 11 and 12 (Scheme 3). These compounds absorb in the NIR region of the electromagnetic spectrum: λ_{max} 778 nm for **11** (in methanol) and λ_{max} 790 nm for **12** (in methanol).

Scheme 3



Anhydrous *N,N*-dimethylformamide (DMF)
Phosphorus oxychloride (caution: corrosive, decomposes to HCl and H_3PO_4 on contact with atmospheric moisture, do not inhale, handle with a syringe)
Cyclohexanone
Aniline
Hydrochloric acid (conc.)
Sodium acetate (anhydrous)
Benzenethiol or thiophenol: Density 1.07 g/ml
Absolute ethanol, dichloromethane, ether (solvents)

Techniques: Crystallization, chromatography, ^{1}H NMR, ^{13}C NMR, NIR spectroscopy

Vilsmeier-Haack reagent 10. Phosphorus oxychloride (1.1 ml, 12 mmol) was added to a 25-ml two-necked flask containing anhydrous *N,N*-dimethylformamide (1.3 ml, 17 mmol) and a magnetic stirring bar. A reflux condenser capped with a drying tube (CaCl2 or Na_2SO_4) was placed in one neck and the second neck was closed with a glass stopper. The mixture was heated under reflux for 1 hour, then cooled and treated dropwise through the condenser with a mixture of aniline and ethanol (1:1, 1.8 ml). Stirring was continued for an additional 30 min after the aniline addition, and then the deep-purple solution was poured onto a mixture of crushed ice (10 g) and conc. hydrochloric acid (1 ml). After the mixture was kept for 2 hours in an ice-bath, the crystalline product **10** was filtered, washed on the filter with cold water and ether, and dried in a desiccator under a reduced pressure; yield 0.XX g (87%), mp 220-221 ^{o}C.

NIR cyanine dye 11.[14] A solution of indolium salt 7 (0.60 g, 2 mmol), Vielsmeyer reagent 10 (0.36 g, 1 mmol), and anhydrous sodium acetate (0.33 g, 4 mmol) in absolute ethanol (30 ml) under a nitrogen atmosphere was heated under reflux for 6 hours. After removal of ethanol on a rotary evaporator, the residue was dissolved in dichloromethane (30 ml) and filtered. The filtrate was treated dropwise with ether, which caused precipitation of

dye **11**. The mixture was kept for 30 min in the refrigerator, and then the dye was filtered, washed on the filter with ether, and dried in a desiccator under a reduced pressure; mp > 250 ^{o}C (dec), λ_{max} 778 nm (in methanol).

NIR cyanine dye 12. A solution of dye **11** (61 mg, 0.1 mmol) and benzenethiol (110 mg, 0.103 ml, 1 mmol) in anhydrous *N,N*-dimethylformamide (5 ml) under a nitrogen atmosphere was stirred at room temperature for 30 min. Concentration of the mixture on a rotary evaporator followed by silica gel chromatography of the residue eluting with dichloromethane/methanol (95:5) gave analytically pure dye **12**; mp > 250 ^{o}C, λ_{max} 790 nm (in methanol).

Post-synthetic assignments

1. Analyze purity of the products **11** and **1**2 by TLC. A mixture of methanol and ether or chloroform is the suggested eluent because these cyanines are highly polar.
2. Record and analyze ^{1}H NMR and ^{13}C NMR spectra of the products **11** and **12** for solutions in DMSO-d_6.
3. Record NIR absorption spectra of the cyanines **11** and **12** in methanol and compare λ_{max} with the provided values.
4. Record ^{1}H NMR spectrum of the substrate **10** in DMSO-d_6 solution and determine which tautomer is present (fully conjugated amino-imine vs. bis-imine).
5. Name compounds **10** and **12** according to the IUPAC approach. References[52-57] contain names of similar dyes. The name of **11** is 2-[4'-chloro-7'-(1'',3'',3''-trimethylindolin-2''-ylidene)-3',5'-(propane-1''',3'''-diyl)-1',3',5'-heptatrien-1'-yl]-1,3,3-trimethyl-3*H*-indolium iodide.[14] Practice by correlating the name with the structure.
6. Discuss the $S_{RN}1$ mechanism for the transformation **11** → **12**. It is described in refs.[53,56]

CYANINE DYES: References

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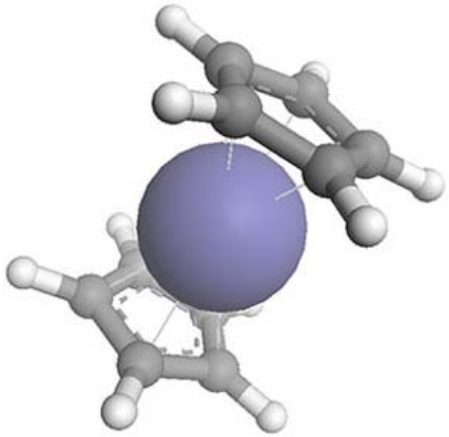
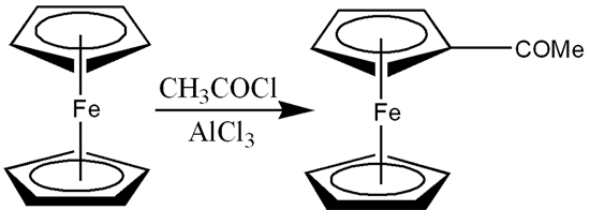
INORGANIC SYNTHESIS

Ferrocene[1] (example of metallocenes)

Properties and reactivity. Ferrocene is an organometallic compound with the formula $Fe(C_5H_5)_2$, in which the central ferrous ion (Fe^{2+}) is sandwiched between two cyclopentadienyl anions ($C_5H_5^-$). Ferrocene is an example of a general class of organometallic compounds called metallocenes. The structure of ferrocene was correctly reported for the first time by E. Fischer and G. Wilkinson (Imperial College, London) who were awarded the Nobel Prize in 1974 for this achievement. Ferrocene is a yellow-orange solid that is highly stable in air. It undergoes melting without decomposition, mp 172-174 ^oC, and can be distilled under atmospheric pressure, bp 249-250 ^oC. Its purification typically involves sublimation under atmospheric pressure. It is soluble in organic solvents but insoluble in water.



The two cyclopentadienyl ligands in ferrocene have 6 π-electrons each, making them aromatic, and the central ferrous ion Fe^{2+} contains 24 electrons (26 – 2 = 24) for a total number of 36 electrons as in noble gas krypton. As a result, ferrocene is a stable molecule (see above) and undergoes many reactions typical of aromatic compounds – for example, Friedel-Crafts acylation.



Uses. Ferrocene itself has found few applications but there are many uses of ferrocene derivatives. It has been used as an additive to a diesel fuel to prevent soot formation and as an

additive to gasoline as an anti-knocking agent, replacing tetraethyl lead. It is also an additive to unleaded gasoline for vintage cars that were originally designed for a lead-containing fuel. Derivatives of ferrocene are used in pharmaceutical and materials chemistry.

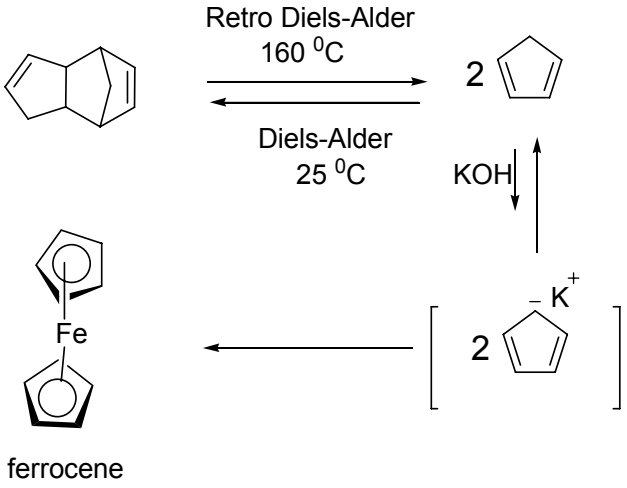
Synthesis of ferrocene

Dicyclopentadiene (or cyclopentadiene dimer). The systematic IUPAC name is 4,7-methano-3a,4,7,7a-tetrahydroindene; bp 170-171 ^{o}C (with decomposition to cyclopentadiene)
Dimethoxyethane (or glyme), dimethyl sulfoxide: solvents
Potassium hydroxide (base), **ferrous chloride tetrahydrate** ($FeCl_4$•$4H_2O$)

Techniques: Distillation, sublimation, mp

The chemistry is shown in Scheme 1. In the first step, cyclopentadiene is produced by thermal cracking dicyclopentadiene, a commercial product. This is efficiently achieved on a distillation still containing an efficient distillation column. Specifically, dicyclopentadiene, bp 170-171 ^{o}C, is heated to 160 ^{o}C in a flask equipped with a distillation column. Cyclopentadiene, bp 40-42 ^{o}C, is slowly distilled off. The product should be collected in a receiving flask cooled with ice to minimize its Diels-Alder dimerization back to dicyclopentadiene. When kept at 0 ^{o}C, cyclopentadiene thus obtained should be used within 2 hours.

Scheme 1



ferrocene

Among simple hydrocarbons, cyclopentadiene is relatively acidic (pK_a= 15.5) because deprotonation generates aromatic 6π-electron cyclopentadienide anion. The reversible ionization can be achieved using strong bases such as potassium hydroxide. The choice of solvent is important and two suitable solvents are 1,2-dimethoxyethane (glyme) and dimethyl sulfoxide (DMSO). These are polar aprotic solvents that favor dissociation of potassium hydroxide into potassium and hydroxide ions. Small amount of water is acceptable, e.g. water introduced with ferrous chloride tetrahydrate, ($FeCl_2$•$4H_2O$), but any additional amounts may greatly decrease basicity of hydroxide ion by forming less reactive hydration complexes (the

more reactive free hydroxide ion is called a naked anion). Accordingly, the solvents used should be at least pre-dried. Even under optimized condition, the concentration of cyclopentadienide anion, generated in reversible deprotonation of cyclopentadiene, is small. A sufficient amount of time should be allowed for the subsequent reaction of cyclopentadienide anion with ferrous cation to produce ferrocene. Note that in Scheme 1 the structure of potassium cyclopentadienide is placed is brackets indicating that this is an intermediate product that cannot be isolated from the mixture.

Experimental Procedure

Cracking of dicyclopentadiene. The cracking apparatus is a simple fractional distillation set-up (lab assistant will help you with the set up) consisting of a 250-ml or 500-ml round bottom flask equipped with a magnetic stirring bar, a distillation column, a thermometer placed at the top of the column, and an efficient water-cooled condenser. The receiving flask should be placed in an ice-bath. The main flask should be filled with dicyclopentadiene to no more than half of the total volume. The flask is placed in a heating mantle and the content is stirred using a magnetic stirrer. A bottle of dicyclopentadiene will be available in one of the hoods in the laboratory. The dimer undergoes cracking slowly on heating and the monomer begins to distill steadily in the 40 - 42°C range. It may take up to 2 hours to distill about 100 ml of cyclopentadiene. One distillation should be set up to serve several students.

Synthesis of ferrocene. In order to not waste time, it is essential to conduct the synthesis as follows. First, ferrous chloride tetrahydrate is grounded in a mortar to a fine powder, and the powdered salt (6.5 g) is placed in a 50-ml Erlenmayer flask equipped with a magnetic stirring bar. The flask is filled with nitrogen by immersing a tubing connected to a nitrogen line (caution: a fast flow of nitrogen may pick up some of the powdered salt), treated with dimethyl sulfoxide (25 ml), and capped after removal of the nitrogen tybing. The mixture is placed on a magnetic stirrer and stirred until a solution is obtained. It should take no more than 10 min to obtain the solution provided the salt is adequately grounded into a fine powder. Then, potassium hydroxide is grounded as rapidly as possible (30 sec?) in a mortar and the fine powder thus prepared is rapidly weighed (30 g) and added to a round-bottom 250-ml flask filled with nitrogen and equipped with a magnetic stirring bar. Do not try to weigh exactly 30 g of KOH because the time of the preparation is of the essence – the longer the time, more water will be absorbed. Alternatively, a dry-box filled with a dry nitrogen can be used for the preparation of powdered KOH. ***Caution:*** *KOH is an irritant – wear gloves.* 1,2-Dimethoxyethane (60 ml) is added to the flask with grounded KOH and the mixture is stirred under a nitrogen atmosphere and treated dropwise with cyclopentadiene (5.5 ml, XX mmol) using a syringe, and the mixture is stirred for an additional 15 min. At this point a nitrogen tubing is removed from the flask and the flask is closed with a rubber septum. The content of the flask is kept under a nitrogen atmosphere with the help of a needle connected to the nitrogen line. The mixture will turn brown in color because of the formation of potassium cyclopentadienide. A solution of the ferrous salt in DMSO is now added dropwise to the flask using a syringe. The entire DMSO solution is added within 40 min and then the dark green mixture is stirred for an additional 30 min. Finally, the mixture is poured onto a crushed ice (100 g) with hydrochloric acid (6M HCl, 90 ml). The slurry is stirred for about 15 min and the

resultant orange precipitate of ferrocene is collected on a Buchner or Hirsch funnel and washed with water (4 X 25 ml). The moist solid is spread out on a large watch glass and dried in the air until the next lab period. Drying can be accelerated by placing the crude product in an oven preheated to 80 ^{o}C. Higher temperatures may result in loss of ferrocene due to sublimation. The dry compound is then purified by sublimation in a large glass Petri dish that is placed on a warm hot plate (100 – 140 ^{0}C). Care is used to avoid melting. Pure ferrocene will collect on the cool upper part of the dish as beautiful yellow crystals. The slower the sublimation process (low temperature) the larger and more elegant crystals will appear. In order to accelerate the purification process, the student may use only part of the crude product for sublimation and then calculate the total yield using the corresponding weights (total crude material and the portion used for sublimation).

Post-synthetic assignments

1. Calculate the yield and measure mp of the purified product.

2. Record ^{1}H NMR spectrum of ferrocene for a solution in $CDCl_3$ or DMSO-d_6. Correlate the spectrum with the structure.

Ferrocene: references

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Tetraphenyltin[1]

Tetraphenyltin is synthesized by the following reactions:

$$2Na + C_6H_5Cl \rightarrow [C_6H_5Na] + NaCl \quad (1)$$

$$4[C_6H_5Na] + SnCl_4 \rightarrow (C_6H_5)_4Sn + 4NaCl \quad (2)$$

In the first step (Equation 1), metallic sodium is allowed to react with chlorobenzene to generate phenylsodium. Then, the reaction of phenylsodium with tin tetrachloride yields the desired product (Equation 2). Note that the symbol of phenylsodium is given in brackets, which indicates that this organometallic reagent is an intermediate product that is not isolated for the subsequent reaction. The first transformation is a heterogeneous reaction between metallic sodium and an organic substrate. Toluene is a solvent. In order to accelerate the formation of phenylsodium, sodium metal must be in the form of a fine sand with a large surface area. Since phenylsodium is moisture sensitive, the synthesis must be conducted under an inert atmosphere of nitrogen. Note from the two equations that a large amount of sodium chloride is a byproduct. This will be seen as a white precipitate which will be an indication that the reaction proceeds well. A final note is on tin tetrachloride. You may be surprised at first to learn that this inorganic chloride is a liquid at room temperature because other chlorides, such as sodium chloride, are solids. Well, everything is relative and even sodium chloride is a liquid at the temperatures above 801 ^{o}C. Anhydrous tin tetrachloride is moisture sensitive. It is supplied in bottles capped with a rubber septum and should be handled with a syringe. For withdrawal of this reagent from the bottle using a syringe, a needle from a nitrogen line should be inserted first through the septum in order to replace the taken-up liquid by an equal volume of nitrogen. See the section *Organolithium Reagents* for additional information. Liquid tin tetrachloride reacts rapidly with atmospheric moisture to yield solid tin tetrachloride pentahydrate ($SnCl_4 \bullet 5H_2O$). You will observe quite a spectacular effect as white fumes are formed around the tip of the syringe filled with the liquid reagent. Old bottles of tin tetrachloride may contain crystalline hydrate at the bottom. The liquid part of the reagent may still be used for the preparation of tetraphenyltin.

Tetraphenyltin and tetraalkyltin analogs are important intermediates for the preparation not only of other tin compounds but also of derivatives of other elements. Important tin compounds are triphenyltin chloride and tributyltin chloride (Equation 3) and the corresponding hydrides (Equation 6).

$$3R_4Sn + SnCl_4 \rightarrow 4R_3SnCl \quad (3)$$

R = Ph or Bu

$$R_3SnCl + NH_3 + H_2O \rightarrow R_3SnOH + NH_4^+ + Cl^- \quad (4)$$

$$2R_3SnOH \rightarrow R_3Sn\text{-}O\text{-}SnR_3 + H_2O \quad (5)$$

$$R_3Sn\text{-}O\text{-}SnR_3 \xrightarrow{LiAlH_4} 2R_3SnH \qquad (6)$$

The chlorides (Equation 3) can be used in the synthesis of stable tetrasubstituted tin compounds, the reaction of which with *n*-butyllithium provides a convenient method for the generation of other lithium reagents. The hydrides (Equation 6) are powerful reducing agents, the reaction of which involves free radicals.

Experimental procedure

Anhydrous toluene
Sodium
Chlorobenzene
Anhydrous tin tetrachloride

Techniques: Azeotropic drying of solvents, generation of phenylsodium, crystallization

Drying of toluene. The preparation involves water-sensitive reagent and, as such, must be conducted in an anhydrous solvent (toluene). This solvent can be dried by using azeotropic distillation. Specifically, distillation of toluene will give the first fraction as a white emulsion of water with toluene because more water is accommodated in the vapor phase than in the liquid mixture. Distillation is continued until the emulsion is not observed any longer; the residue that was not distilled is dry enough for the synthesis of tetraphenyltin.

Preparation of a sodium sand. Sodium is stored in a jar filled with a mineral oil. A small beaker containing a 1/3 volume of dry toluene is zeroed on a two-digit electronic balance. A small chunk of sodium is removed from the storage jar using forceps, placed on a dry napkin, quickly wiped with another napkin, and added to the beaker on the balance. This procedure should be repeated several times until 5 g of sodium is weighed. ***Caution:*** *do not put the used napkins in the sink or in the trashcan because fire may start from the remaining traces of sodium on contact with water. Sodium should be neutralized by the reaction with ethanol*. Use common sense: do not try to weigh exactly 5.00 g. Any amount in the range of 4.5 g – 5.0 g is acceptable. Then transfer the sodium chunks and toluene from the beaker to a 250-ml three-neck flask equipped with a large magnetic stirring bar and a reflux condenser in one of the necks. Then add more toluene to about 100 ml, place the setup in the heating mantle sitting on a magnetic stirrer, and start the stirring. **Note:** *no water is allowed in the condenser*. While stirring gently, place a nitrogen tubing in the condenser to remove air from the flask, then close the remaining two necks of the flask with glass stoppers (see the comment in the section *Lithium Reagents*), and heat the flask until sodium is melted: bp of toluene is 110 ^{o}C, mp of sodium is 97.8 ^{o}C. With sodium in the liquid state increase the rate of stirring until a fine liquid droplets are formed. Quickly remove the heating mantle and continue stirring until the temperature decreases below mp of sodium and a finely dispersed sodium sand is formed. No piece of sodium should be larger than 1 mm in diameter. If you do not succeed at the first attempt, try all over again. And then again and again…

Generation of phenylsodium. With sodium sand successfully prepared and the mixture cooled to room temperature, one stopper is replaced by a thermometer and a second one by a rubber septum. A tubing with nitrogen is removed from the condenser, and the opening is closed with another rubber septum. A needle connected with a nitrogen line is inserted into the septum to maintain inert atmosphere and equalize the pressure. Then the sodium dispersion is heated to 45 ^{o}C, stirred gently, and treated dropwise with chlorobenzene (12 ml – calculated for 5 g of sodium; adjust if a different amount of sodium was weighed) using a syringe. A volume of about 1 ml may be added at once but caution should be observed afterwards because the reaction of sodium with chlorobenzene is exothermic. If you use a two-neck flask, the septum at the top of the condenser may be used to add chlorobenzene. The addition may take up to 1 hour during which time the temperature in the flask should not exceed 55 ^{o}C. Higher temperatures favor the formation of biphenyl by the reaction of phenylsodium with chlorobenzene. Maintaining the temperature below 55 ^{o}C must be aided with a non-aqueous bath. Typically a kerosene bath should be used. The addition of small pieces of dry ice (solid carbon dioxide) will lower the temperature of the bath.

Synthesis of tetraphenyltin. After all the chlorobenzene has been added (about 1 hour), place a solution of 3.4 ml of tin tetrachloride (calculated for 5 g of sodium) in 8 ml of dry toluene in a syringe and, over a period of 30 min, add dropwise this solution to the reaction flask. During this addition, it is necessary to cool the flask so as to keep the temperature below 45 ^{o}C. The flask may now be stored indefinitely under an air atmosphere. **Caution:** read about glass-on-glass connections under basic conditions in the section *Organolithium Reagents*. You may leave the flask until the next week. For isolation of tetraphenyltin stir gently and heat the mixture on a heating mantle up to 80 ^{o}C to dissolve the product and then filter the content using a preheated filter. Place the solid back in the flask, add toluene (50 ml), heat the mixture and filter it again. This extraction of tetraphenyltin should be performed one more time. The combined extracts are cooled in an ice bath, the resultant crystals of the product are filtered, and the filtrate is concentrated on a rotary evaporator, which yields an additional amount of the product. After crystallization from toluene the yield is about 8 g, mp 228 – 229 ^{o}C.

Post-synthetic assignments

1. Calculate percent yields for the procedure above and your experiment, determine mp.
2. Obtain and analyze the ^{1}H NMR spectrum of tetraphenyltin.
3. What are the products of the reaction of phenylsodium with D_2O?
4. Why water cannot be used in the condenser for the experiment described above?

Tetraphenyltin: references

1. W. J. Jolly, *The Synthesis and Characterization of Inorganic Compounds*, Waveland Press, Inc., 1991, p. 475.