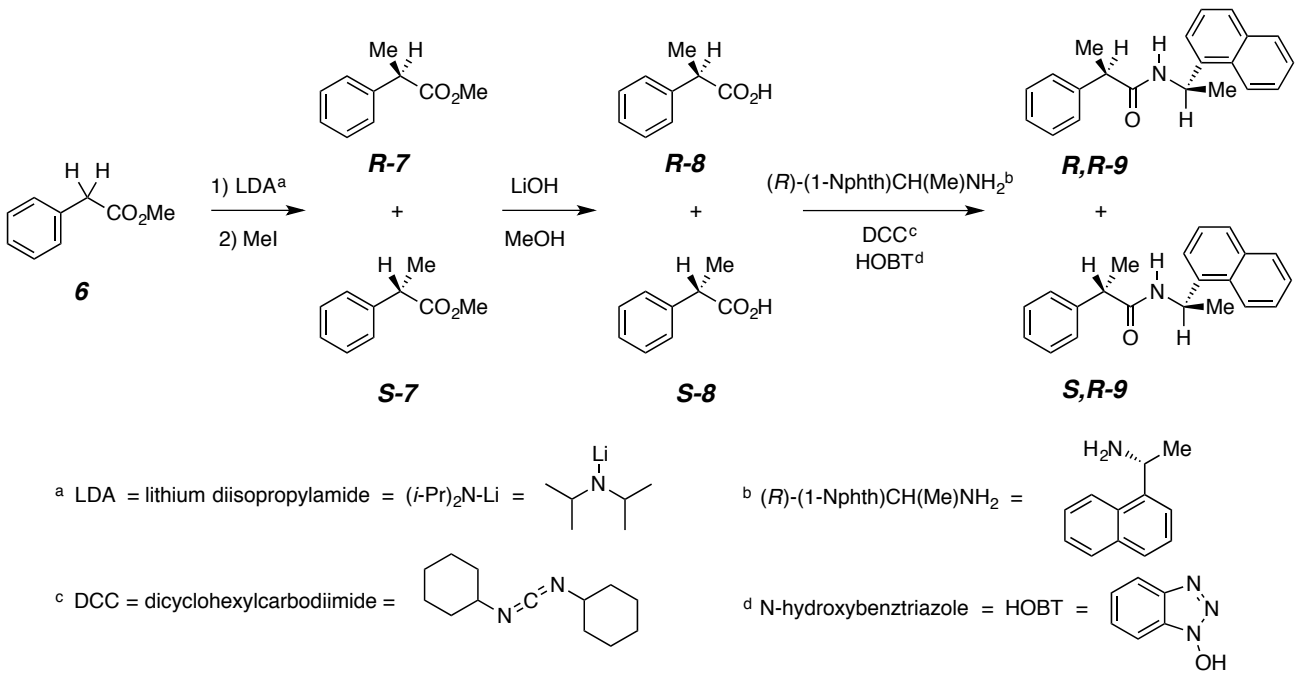
Experiment 3
Enolate Alkylation, Ester Saponification, and Amide Bond Formation via DCC-Coupling

*Enolate alkylation (**6** to **7**), ester hydrolysis (**7** to **8**), and DCC/HOBT-coupling (**8** to **9**) in the preparation of* N*-1-(1-naphthyl)ethyl α-methylphenylacetamides (**9**).*

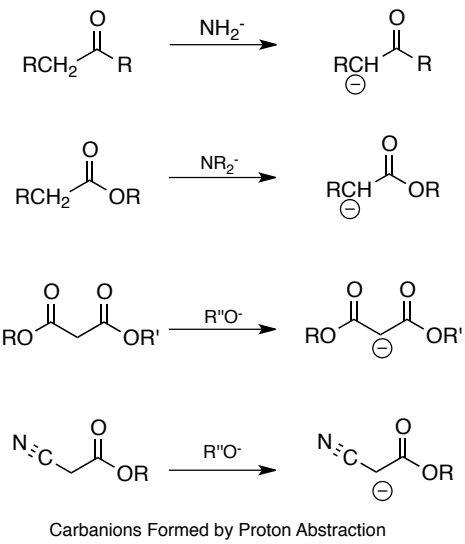
Reaction Sequence:



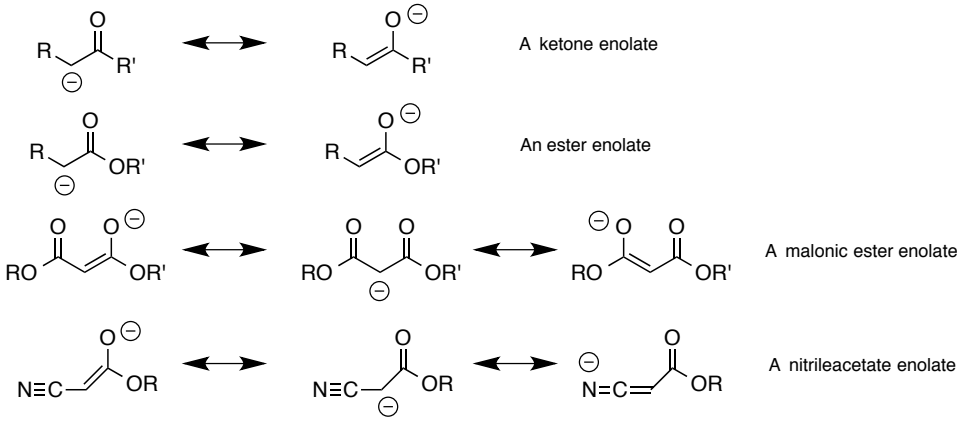
Background

As you may know/recall, the carbonyl group plays a central role in organic synthesis. Various nucleophiles can be added to the carbonyl carbon atom. For example, reduction of the carbonyl group by hydride ion gives an alcohol (as you have done in Experiment #1), and addition of carbanionic species (e.g., Grignard reagents) is a valuable method for building up carbon skeletons.

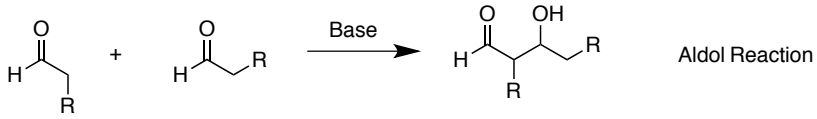
The α-carbon to a carbonyl compound is also a site of many important reactions. This is in large part because the protons α to a carbonyl group are acidic, a characteristic on which you capitalized for the equilibration of menthone and isomenthone in Experiment #2. The pK_a values for α-protons range from ~9, for α-protons of a β-dicarbonyl compound, to ~25 for alpha protons of an aliphatic ester. The typical pK_a of a ketone α-proton is ~20. Since α-protons are weakly acidic, they can be removed with a very strong base to form an enolate anion. Most of the negative charge (i.e., the extra electron density) of this species resides on the oxygen atom.



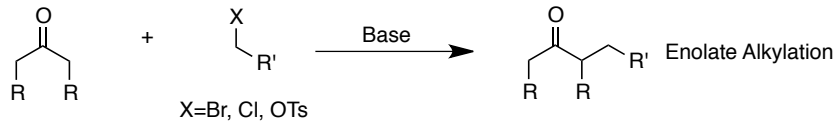
Carbanions Formed by Proton Abstraction



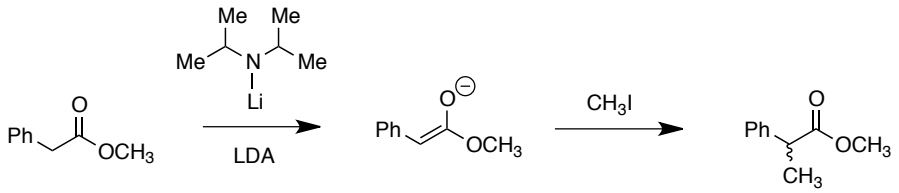
Once generated, an enolate ion can undergo a variety of reactions. Below is a classic transformation known as the aldol addition reaction, since the product is a β-hydroxyaldehyde (i.e., an "ald-ol").



Reaction of the enolate with an alkyl halide is referred to as alkylation. The leaving group can be, e.g., I^-, Br^-, or tosylate anion. It is this transformation that you will perform as the first step in the multistep sequence in Experiment 3.



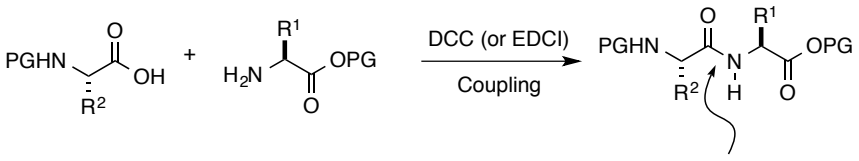
The ester starting material that you will use is methyl phenylacetate (**6**). Deprotonation of this ester by lithium diisopropylamide (LDA) will give the enolate **6**$^-$. Once the enolate is formed, you will add methyl iodide (MeI) and alkylate **6**$^-$ in an S_N2 reaction. The product of this reaction, methyl (±)-2-phenylpropanoate (**7**), will be formed as a racemic mixture.



6 ***6*** $^{-}$ ***(±)-7***

You will then synthesize a mixture of the *diastereomeric* amides ***R,R*-9** and ***S,R*-9** by reaction of racemic 2-phenylpropanoic acid (**8**) with the optically pure amine, (*R*)-Ph(Me)CHNH$_2$. These diastereomeric amides will then be separated by MPLC. In the process of forming these amides, you will use a very important reaction, the formation of the "peptide bond" (a carboxylic amide) using dicyclohexylcarbodiimide (DCC) as the activating agent.

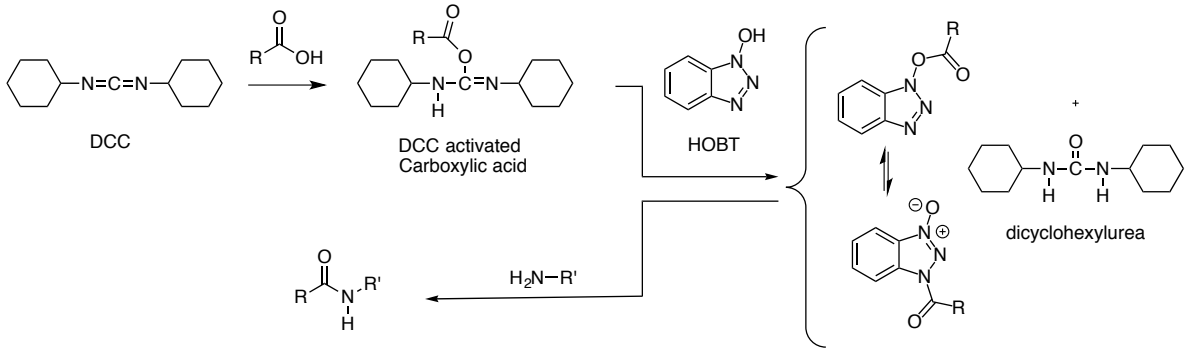
The formation of peptides and proteins from amino acids is an important process in bioorganic chemistry. A peptide, which is a polyamide containing ≤ ~100 amino acid monomer units and is a substructure of a protein, a larger biopolymer that typically contains ≥ ~100 amino acids, can be made by sequentially reacting the amine portion of an α-amino acid with the carboxyl group of a second α-amino acid of the growing peptide chain.



PG = protecting group Peptide bond

One of the most common methods for activation of an acid is the use of carbodiimides, often, dicyclohexylcarbodiimide (DCC). For mechanistic reasons and to minimize potential byproduct formation, this reaction is often performed in the presence of 1-hydroxybenztriazole (HOBT). This generates acylated HOBT adducts that are the activated species that react with the amine.

[BTW, for NMR spectra of acylated HOBT adducts (and precursor DCC/HOBT adducts), see: "Synthesis of a putative subtype specific antigenic heptapeptide from *Escherichia coli KBB ad* protein fimbriae." Meldal, M. *Acta Chem. Scand. B* **1986**, *40*, 242-249.]



Once the acid is activated, it reacts with the amine to form the amide bond. You will first need to hydrolyze your racemic methyl 2-phenylpropanoate (**7**) with the base LiOH in *aqueous* methanol. The product carboxylic acid **8** will then be coupled with (*R*)-(+)-1-(1-naphthyl)ethylamine using DCC/HOBT.

Experimental Design:

Methylation of methyl 2-phenylacetate (6) with methyl iodide:

Devise a procedure for preparing a solution of lithium diisopropylamide (LDA)[1] to react with methyl phenylacetate ($PhCH_2CO_2Me$, **6**). Start with ~5 mmol of the ester. The concentration of the enolate anion in THF should be about 0.5 M. The reaction should be performed under a nitrogen atmosphere. Use *anhydrous* THF (from a small reagent bottle clamped in the hood). Since at least small amounts of the strongly basic species will unavoidably be quenched by trace amounts of proton sources (e.g., water on the walls of the flask and in the "anhydrous" solvents), you should plan to use a slightly higher excess of base (lithium diisopropylamide, ~1.25 mmol for each mmol of ester). Check the titer listed on the commercial bottle of *n*-BuLi in hexanes (also clamped in the hood) to calculate the volume of *n*-BuLi solution that should be added. You will need to use proper syringe-handling techniques for dispensing the potentially flammable *n*-BuLi solution. Use only a slight excess of methyl iodide in the alkylation. Once the alkylation is finished, quench the reaction mixture with NH_4Cl (satd) and dilute with ether. Workup the reaction mixture in the usual manner. *Purify the methylated ester product by flash column chromatography or MPLC (your choice) and characterize by the usual battery of spectroscopic techniques*. (Use the larger (ca. 2.5 cm diameter) MPLC column if you choose that option.) Separation of the methylated product(s) from starting material and from a dimethylated byproduct is challenging, but doable. We will assess the level of purity of this product, in part, by the extent to which you isolate it free of the starting material and/or the dimethylated byproduct.

Caution: Methyl iodide is volatile and fairly toxic because it is a reactive electrophile (an alkylating agent); it should only be handled in the hood. Measure and dispense it by syringe, using its density to convert mmol to mass to volume. It is imperative that you understand (and execute) how to precisely dispense the proper volume of liquid in this transfer.

Hydrolysis (saponification) of methyl 2-phenylpropanoate (7):

In an appropriately sized culture tube, dissolve the ester **7** in ~2-5 mL of methanol. Add water dropwise until the mixture first becomes just slightly cloudy or until an equal volume of water has been added. Add 2 equivalents of K_2CO_3 or $LiOH{\bullet}H_2O$ and allow the mixture to stir at ambient temperature or warm it to ca. 50 °C in a water bath. When the reaction is complete (monitor by TLC, look for disappearance of starting ester), cool to room temperature, and dilute with ether. Adjust the pH of the water layer to pH <3 with 10 % HCl. Extract the acidic water layer with ether. Wash the combined ether extracts with brine, dry them over $MgSO_4$, etc. Characterize your **crude** acid by the usual, full battery of spectroscopic techniques, but **do not purify** it on SiO_2. *This is the only product in this course that you will not purify*. Carboxylic acids sometimes behave poorly upon attempted chromatographic purification (e.g., streak on TLC or band-broaden during flash chromatographic or MPLC purification).

DCC coupling with (*R*)-(+)-1-(1-naphthyl)ethylamine

Couple the racemic acid **8** with (*R*)-(+)-1-(1-naphthyl)ethylamine using dicyclohexylcarbodiimide (DCC) as the activating/dehydrating agent. *Caution: Some people become sensitized to DCC if they come in contact with the compound*. Also carbodiimides react slowly with moisture. For these reason*s* we have prepared a solution of DCC in dichloromethane (DCM) for you to use. Please ask a TA to help you with dispensing the volume of that stock solution that you would like to use. The titer (i.e., molarity) will be labeled on the container, which will be a round-bottom flask fitted with a septum clamped in the hood. Minimize exposure of your reaction mixture to atmospheric moisture, throughout, by flushing the headspace of your reaction vessel with nitrogen.

In your reaction vessel, place the acid **8** and 1.1 equivalents of HOBT (1-hydroxybenztriazole). Add enough ethyl acetate, the reaction solvent, to bring the molarity of the mixture to ca. 0.2 M. The acid will fully dissolve

but the HOBT will remain in suspension; it is only sparingly soluble in many organic solvents, including EtOAc (and DCM and $CHCl_3$). Prepare a separate solution of (*R*)-(+)-1-(1-naphthyl)ethylamine (1.5 equiv) and triethylamine (1.5 equiv) in EtOAc. Add to your main reaction vessel (by syringe) 1.1 equivalents of the DCC stock solution in dichloromethane (DCM) that the TAs have prepared in a Schlenk flask. The concentration (molarity) of that solution will be labeled on the Schlenk flask containing that solution. You may notice that the suspension changes in consistency or appearance because the HOBT becomes acylated by the carboxylic acid and one equivalent of (the sparingly soluble) dicyclohexylurea (DCU) is produced. Neither the HOBT nor the DCU have good solubility in DCM. You will not be able to monitor change by tlc, because the activated acid species are likely not stable to silica gel. Add your solution of the naphthylethylamine (by syringe) to the stirred reaction mixture once you visually detect or sense no further change in the composition of the suspension.

Monitor reaction progress by tlc. When you have deemed that the reaction is complete (tlc), filter the entire mixture through a short column of Celite. Much of the byproduct DCU and (regenerated) HOBT will be out of solution. Rinse the column with a small amount of ether (you may(?) see some additional precipitate appear in the filtrate, which is fine given the next operation). Evaporate the entire filtrate to dryness. Add ca. 10 mL of extraction ether and mix well with a spatula and swirling. DCU is far *less* soluble in ether than in DCM. Your product amides will dissolve in this ether, any remaining DCU will not. Filter once again through Celite (you can use the same column bed as before). Wash the filtrate ether solution with 10% HCl (to remove any remaining amines as water-soluble ammonium ions) and brine, dry the ether solution with $MgSO_4$, filter, and evaporate. This material, ideally without any observable amount of remaining DCU, is your crude mass recovery. Separate the pair of diastereomeric amides **9** by MPLC* (or careful flash chromatography; this is a challenging, but doable, flash separation). These are only marginally soluble in a hex:EtOAc mixture of appropriate polarity. Therefore, we recommend suspending the crude sample in the elution solvent and adding methylene chloride dropwise until the mixture becomes homogenous. Loas that solution onto the MPLC injector loop. If there is still solid remaining after having added an equivalent volume of DCM, that is likely DCU. Do NOT attempt to load this suspension onto the MPLC (the solid will clog the injector body – a big no-no). Either attempt the separation on a flash column, which is readily achievable, or filter the sample through a short plug of silica gel (elution with 1:1 hex:EtOAc) to remove that remaining DCU and then proceed as above.

Collect spectral data on each pure diastereomer. Allow your samples to dry at least overnight before measuring the melting point (mp). Always record and report a temperature *range* for a mp, not a single value.

The following data were collected by previous TAs, principally Dorian Sneddon:

Diastereomer 1	**Diastereomer 2**
(elutes faster on SiO_2)	(elutes faster on SiO_2)
mp = 155–156 °C	mp = 159–160 °C
GC t_R = ca. 14.9 min	GC t_R = ca. 14.7 min
^{1}H NMR ($CDCl_3$) includes:	^{1}H NMR ($CDCl_3$) includes:
two doublets, 3H δ = 1.51 and 1.54 ppm	two doublets, 3H δ = 1.52 and 1.59 ppm
x-ray diffraction analysis, performed (Oct. 2020)*	

* to open and view on a browser the .pdb file of this x-ray structure, which has been uploaded to the website, open: https://www.ncbi.nlm.nih.gov/Structure/icn3d/

Ignore the small window titled “Please input …”

Instead, under the “File” dropdown, select “Open file” and then “PDB File (appendable).”

In the small window you can now browse to the location on your computer of the .pdb file named “20100z Amide X-ray Structure Inverted in Mercury enantiomer with R amine.pdb” that I have placed on the course website and that you should download to your computer.

Lab Report Questions (Please answer in your own words):

1. Rank the following ketones from highest R_f to lowest R_f on tlc: a) cyclohexanone, b) 2,2-dimethylcyclohexanone, and c) 2-methylcyclohexanone.
2. Why is it advantageous for the atmosphere inside the reaction flask during the ester enolate alkylation reaction be inert (e.g., dry nitrogen rather than lab air)?
3. What class of reaction mechanism is the alkylation (methylation) reaction? Why is methyl iodide used instead of methyl bromide in this experiment? Would you expect *n*-propyl iodide to react faster than, slower than, or at the same rate as *i*-propyl iodide (2-iodobutane) in an analogous reaction? Explain.
4. What product is formed upon the reaction of EDCI (look up the structure of this amide bond coupling reagent), an analog of DCC, with moisture in the atmosphere?
5. In the basic hydrolysis of the ester **7** to the carboxylic acid **8**, why is it important to add HCl to lower the pH before extracting the product into the organic extraction solvent?
6. Would samples of pure ***R*-8** and pure ***S*-8** have identical or different ^{13}C NMR spectra? Why? Would the sign of the specific rotation (of plane-polarized light) of each be identical or different? Why?
7. Would samples of pure ***R,R*-9** and pure ***S,R*-9** have identical or different IR spectra? Would the absolute value of the specific rotation (of plane-polarized light) be identical or different (or zero)? Why?
8. Amides, like esters, can be hydrolyzed to carboxylic acids. Would you expect ***R,R*-9** and ***S,S*-9** to react at the same or different rates with LiOH/water? Why?

References

[1] E.g., (a) Heathcock, C. H.; Buse, C. T.; Kleschick, W. A.; Pirrung, M. C.; Sohn, J. E.; Lampe, J. *J. Org. Chem.* **1980**, *45*, 1066–1081. (b) Singh, D. K.; Springer, J. B.; Goodson, P. A.; Corcoran, R. C. *J. Org. Chem.* **1996**, *61*, 1436–1442. (c) Wu, G.; Tormos, W. *J. Org. Chem.* **1997**, *62*, 6412–6414.