ISSN 1430-4171
http://journals.springer-ny.com/chedr
10.1007/s00897980205a

Laboratories and Demonstrations

Reductive Amination of Pyruvate Esters: A Microscale Synthesis of *N*-Benzylalanine Esters

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When students work in pairs, structure determination, though challenging, is within the reach of introductory organic students.

The reductive amination of pyruvate esters using benzylamine and sodium triacetoxyborohydride is described. Students isolate the *N*-benzylalanine ester and determine its structure using NMR and IR spectroscopy. Computational methods using CAChe provide information on reactive sites in the pyruvate substrate and reaction intermediates and allow for insight into the reaction mechanism. This experiment is safer than the more traditional reductive amination techniques, and it is appropriate for the introductory organic laboratory.

Reductive amination of aldehydes and ketones is an excellent method for the synthesis of amines and usually receives some coverage in organic chemistry textbooks [1–9]. However, a

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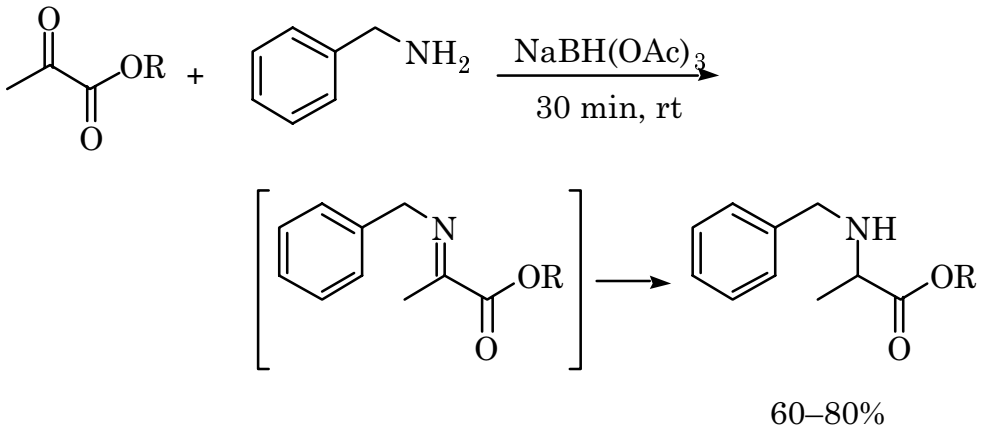

FIGURE 1. REDUCTIVE ANIMATION WITH $NaBH(OAc)_3$.

search of the chemical education literature and some popular organic laboratory texts [10–14] does not reveal any instructional laboratory experiments using this important method. A review of the chemical literature shows reductive amination to be a widely used technique [15]; unfortunately, the toxicity of sodium cyanoborohydride, the preferred reducing agent, precludes the use of these reactions in an undergraduate setting. Recently, however, Abdel-Magid and co-workers [16, 17] described the use of sodium triacetoxyborohydride, $NaBH(OAc)_3$ [18, 19] as a safe and efficient alternative to $NaBH_3CN$ [20]. We describe herein our adaptation of their procedure to the reductive amination of pyruvate esters with benzylamine (Figure 1).

The original work of Abdel-Magid, et al. was quite extensive [16] and provided us with a number of potential carbonyl–amine pairs from which to choose. Our choice of pyruvate esters and benzylamine was based on a number of factors. The reaction times were short enough (30 minutes) to allow easy incorporation of the experiment into a typical laboratory period. Reaction yields were high; students typically obtained yields of 60–80%. The product of the reaction is an *N*-protected alanine ester, a compound of interest to the many biology and biochemistry majors in our course.

Structure determination was made via analysis of the ^1H NMR spectrum of the product. Due to overlap of signals, we found that it was best to have students work in pairs, one using methyl pyruvate and the other using ethyl pyruvate. In both products, the benzylic protons are diastereotopic and appear as two doublets due to the newly created chiral center. The product formed from methyl pyruvate showed (300 MHz) an

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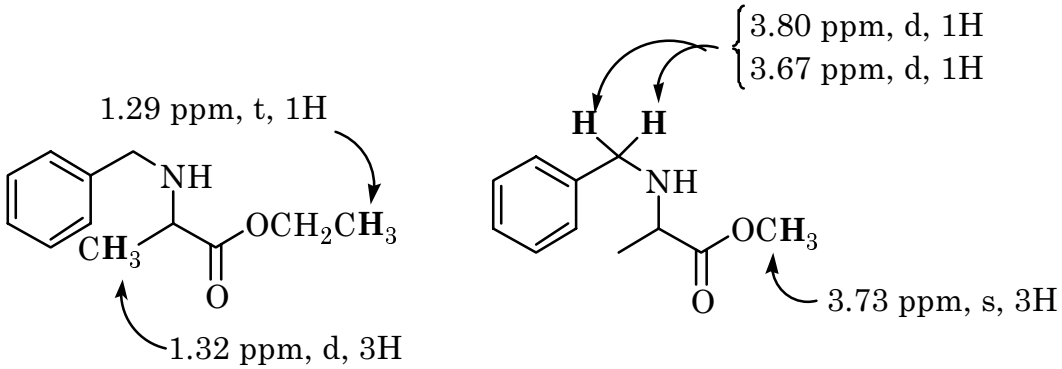

FIGURE 2. COMPARISON OF PRODUCT NMR DATA.

overlap of the signals from the methyl ester singlet and the benzylic signals. The product formed from ethyl pyruvate gave an overlap of the signals from the methyl triplet of the ethyl ester and the doublet from the methyl β to the ester. However, when they compared spectra, the students could "subtract out" the alkoxy signals and structure elucidation was simplified (Figure 2).

Experimental

Methyl pyruvate and ethyl pyruvate were obtained from Aldrich and Lancaster, respectively, and used as received. Sodium triacetoxyborohydride was obtained from Acros and used as received. Benzylamine was obtained from Baker and distilled by a teaching assistant prior to the laboratory session. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 spectrometer at 300 MHz and 75.5 MHz, respectively. Infrared spectra were recorded on a Perkin-Elmer Spectrum 1000 FT-IR spectrometer.

Molecular modeling was performed using CAChe (version 3.7) [21] which calculated nucleophilic susceptibility after optimizing geometry with Augmented MM2 followed by MOPAC [22]using PM3 parameters. Nucleophilic susceptibility "reveals reactive sites based on the electron distribution of a set of active orbitals near the LUMO" [21, 23].

General Procedure

To a 10-mL round-bottom flask containing a magnetic stir bar was added 1.00 mmol of alkyl pyruvate, 1.00 mmol of benzylamine, and 3.5 mL of CH_2Cl_2. The mixture was stirred for approximately 5 minutes, and 1.43 mmol of $NaBH(OAc)_3$ was added in one

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http://journals.springer-ny.com/chedr
S 1430-4171 (98) 03205-8

portion. After stirring at room temperature for 30 minutes, the reaction was quenched by addition of 3.5 mL of saturated $NaHCO_3$. After stirring for 5 minutes, the reaction mixture was transferred to a centrifuge tube, the layers were separated, and the aqueous phase was extracted twice with 1 mL of CH_2Cl_2. The combined organic layers were passed through a plug of anhydrous $MgSO_4$. Solvent was removed, using a rotary evaporator, to yield the alkyl *N*-benzylalaninate as a colorless oil. After recording the mass of the isolated product, an IR spectrum was recorded and a sample was sumitted for NMR analysis.

N-benzylalanine methyl ester (82% yield): ^{1}H NMR ($CDCl_3$, δ): 7.32 (m, 5H), 3.80 (d, J = 12.8 Hz, 1H), 3.73 (s, 3H), 3.67 (d, J = 12.8 Hz, 1H), 3.40 (q, J = 7.0 Hz, 1H), 2.00 (br s, 1H), 1.32 (d, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, δ): 176.1, 139.6, 128.3, 128.1, 127.0, 55.8, 51.8, 51.7, 19.0; IR (neat, cm^{-1}): 3330, 2952, 1736, 1454, 1199, 1152.

N-benzylalanine ethyl ester (85% yield): ^{1}H NMR ($CDCl_3$, δ): 7.29 (m, 5H), 4.19 (q, J = 7.1 Hz, 2H), 3.81 (d, J = 12.8 Hz, 1H), 3.67 (d, J = 12.8 Hz, 1H), 3.37 (q, J = 7.0 Hz, 1H), 2.06 (br s, 1H), 1.32 (d, J = 7.0 Hz, 3H), 1.29 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, δ): 175.6, 139.7, 128.3, 128.1, 126.9, 60.5, 55.8, 51.8, 19.0, 14.2; IR (neat, cm^{-1}): 3329, 2980, 1732, 1453, 1187, 1153.

Discussion

Although most of the experiments in our organic sequence are of a synthetic nature, we have incorporated elements of computational chemistry into a number of experiments as a means of emphasizing differences in substrate reactivity [24, 25]. So, in addition to the synthesis of a protected amino acid and the accompanying structure elucidation, the selectivity of the reagents is a key discussion point in this experiment. Benzylamine reacts exclusively with the ketone carbonyl in pyruvate while $NaBH(OAc)_3$ selectively reduces the imine of the intermediate. The selectivity of both steps of this reaction can be investigated using molecular modeling with CAChe.

In the formation of the imine intermediate, the results (Figure 3) of an assessment of the “nucleophilic susceptibility” clearly show that the ketone carbonyl is much more reactive toward a nucleophile than the ester carbonyl, and formation of the imine thus results. Similarly, once the imine has formed, $NaBH(OAc)_3$ will attack the more susceptible carbon and, again, nucleophilic susceptibility (as determined by CAChe)

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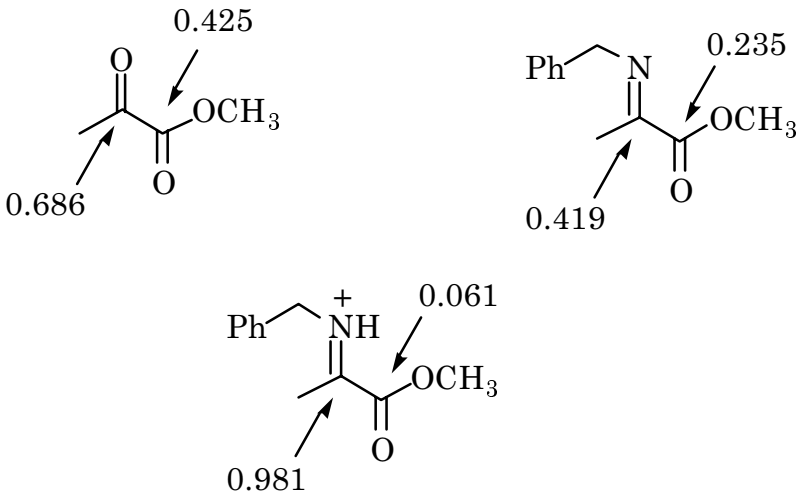

FIGURE 3. CALCULATED NUCLEOPHILIC SUSCEPTIBILITIES.

suggests that the imine will be reduced selectively. However, the CAChe results indicate that, if the imine does not form in the brief period prior to introduction of the reducing agent, reduction of the ketone by $NaBH(OAc)_3$ should compete with reduction of the imine, resulting in a product mixture containing alkyl 2-hydroxypropionate. But, this outcome is not observed. In fact, when $NaBH(OAc)_3$ is mixed with pyruvate prior to the addition of benzylamine, the product is the same as that obtained using the method described in the Experimental Section.

The solution to this paradox lies in the inherent basicity of the imine intermediate. Imines will protonate even when the pH of a reaction is 6–8 and the resultant iminium ion is more susceptible to reduction than the carbonyl of a ketone or an ester [16, 26] Indeed, the iminium ion intermediate is in equilibrium with the imine and it is the protonated species that undergoes reduction (Figure 4).

Sodium triacetoxyborohydride is also an unusually selective reducing agent. While $NaBH_4$ reduces aldehydes and ketones in the presence of esters, $NaBH(OAc)_3$ has been used to reduce aldehydes in the presence of ketones [27]. This additional selectivity has been attributed to the sterically bulky and inductively electron withdrawing acetoxy groups that stabilize the B–H bond [16, 27]. The success of reductive aminations using this reagent is undoubtedly due, in part, to this selectivity. A more powerful reducing agent could be expected to reduce the ketone before the iminium ion intermediate could form.

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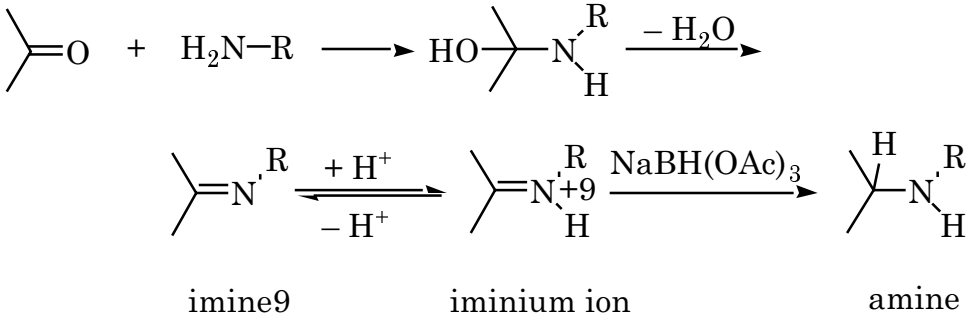

FIGURE 4. REDUCTION MECHANISM.

Conclusion

The reductive amination of pyruvate esters using benzylamine and $NaBH(OAc)_3$ has proven to be an operationally simple experiment that requires students to analyze ^1H NMR and IR spectra and use computational methods to compare the reactivities of ketone and ester carbonyls as well as imines and iminium ions. Chemical yields typically range from 60% to 80%. When students work in pairs, structure determination, though challenging, is within the reach of introductory organic students.

ACKNOWLEDGEMENT

We wish to thank the National Science Foundation for partial funding of Dickinson College's Bruker DPX-300 NMR spectrometer (DUE #9351330) and Molecular Modeling Lab, including CAChe software and accompanying hardware (DUE #9450995).

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