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Article

Spectrophotometric comparison of 4-Nitrophenyl carbonates & carbamates as base-labile protecting groups

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SUMMARY

In organic synthesis, protecting groups are derivatives of reactive functionalities that play a key role in ensuring chemoselectivity of chemical transformations. To protect alcohols and amines, acid-labile tert-butyloxycarbonyl protecting groups are often employed but are avoided when the substrate is acid-sensitive. Thus, orthogonal base-labile protecting groups have been in demand to enable selective deprotection and to preserve the reactivity of acid-sensitive substrates. To meet this demand, we present 4-nitrophenyl carbonates and carbamates as orthogonal base-labile protecting group strategies. These protecting groups are relatively stable in aqueous and acidic solution yet cleaved and irreversibly decarboxylated in mild basic conditions. We showed that deprotection can be monitored spectroscopically, as hydrolysis yields 4-nitrophenol, a bright yellow compound with an optical readout at 413 nm. Finally, we demonstrated that the use of 4-nitrophenol as an effective leaving group allows for deprotection to be carried out in mild conditions. We protected benzyl alcohol and benzylamine via acylation by 4-nitrophenyl chloroformate, yielding substrates that were subsequently subjected to hydrolysis in various pH conditions. The release of 4-nitrophenol was monitored spectroscopically and the reaction kinetics were derived from absorbance data, yielding that hydrolysis was accelerated only in basic conditions and was most effective in pH 12 and above. These results inform the feasibility of our protecting group in organic synthesis and open avenues toward new synthetic routes by broadening the spectrum of effective protecting group strategies.

INTRODUCTION

Synthetic organic chemistry relies on chemoselectivity—the ability to control bond formation events and maximize the yield of favorable products (1, 2). In this regard, appropriate protecting groups are crucial to preventing undesirable side reactions (3-6). Upon attachment, protecting groups inhibit reactivity of the protected functional group and allow for selective bond formation (7). The main criticism of protecting groups, especially in multi-step synthesis, is that they complicate the synthetic route by adding two steps (protection and deprotection), which jeopardize the overall chemical yield and synthetic efficiency (8, 9). Therefore, it is important to identify protecting groups that can be rapidly deprotected with high yield. Additionally, orthogonal protection is advantageous for substrates with multiple functional groups because it enables selective deprotection of one protected group for further reaction, while preserving the other protected groups (10, 11). Thus, protecting groups must meet the following conditions to be synthetically viable options: they must be stable in the given reaction conditions, easily introduced and safely removed in high yield, and positioned orthogonal to pre-existing protected functionalities.

To meet the demand for synthetically applicable protecting groups, we present 4-nitrophenyl carbonates and carbamates as new protecting group strategies that meet the above stated criteria. In this study, we utilized UV-visible spectroscopy to quantitatively compare the hydrolysis kinetics of 4-nitrophenyl carbonates and carbamates as colorimetrically trackable, base-labile protecting groups (**Figure 1**).

4-nitrophenol was chosen because it is a good leaving group (pKa = 7.15 at 25°C), which we hypothesized would allow for deprotection to take place in relatively mild conditions (12). In addition, under basic conditions, 4-nitrophenol exists as a yellow-colored 4-nitrophenolate ion, thus providing a means of spectroscopic confirmation and quantification of deprotection (13). The utility of 4-nitrophenol was applied in our previous study in which we reported the use of 4-nitrophenyl to derive Hammett and Taft plots for enzyme-catalyzed hydrolysis reactions (14-16).

We hypothesized that deprotection would be most effective in basic environments, that the protecting group would remain stable in neutral and acidic conditions, and that the 4-nitrophenyl carbamate would require higher pH

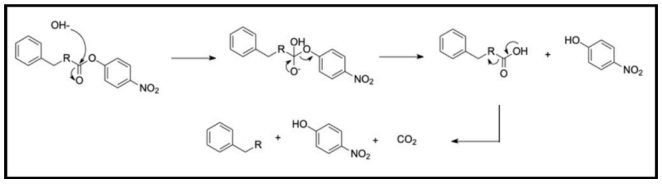

Figure 1: Mechanism of base-catalyzed hydrolysis of the 4-nitrophenyl carbonate and carbamate compounds. The R-group corresponds to the oxygen on the carbonate and the amide group on the carbamate.

conditions to hydrolyze as compared to the carbonate.

To study this protecting group strategy, we protected benzyl alcohol and benzylamine as surrogate substrates. We predicted that 4-nitrophenyl benzylcarbonate would uniformly deprotect at a faster rate than 4-nitrophenyl benzylcarbamate due to differences in charge density surrounding the carbonyl carbon. We found that while 4-nitrophenyl benzylcarbonate indeed deprotected faster than 4-nitrophenyl benzyl carbamate, both proved to be stable in acidic and neutral pH conditions. This demonstrates that both strategies are effective, orthogonal, base-labile protecting groups.

RESULTS

Protecting groups were added via acylation of benzyl alcohol and benzylamine with 4-nitrophenyl chloroformate with 72%-94% yield. Initial attempts to protect benzylamine in the same manner as benzyl alcohol proved unsuccessful, likely due to the increased nucleophilicity of the amine functionality, which may have unfavorably released 4-nitrophenol, causing an immediate change in reaction color to bright yellow. Thus, we adjusted our synthetic approach to counteract this by lowering the reaction temperature and by altering the order of reagents.

These compounds were then subjected to hydrolysis in acidic and basic pH conditions and kinetic data was obtained by spectroscopically monitoring the absorbance at 413 nm. Deprotection could also be observed visually through a change in the reaction color from clear to bright yellow (**Figure 2A**). The rising absorbance at 413 nm at increasing pH values is indicative of deprotection of the substrate since only free nitrophenol absorbs at that wavelength. These spectroscopic

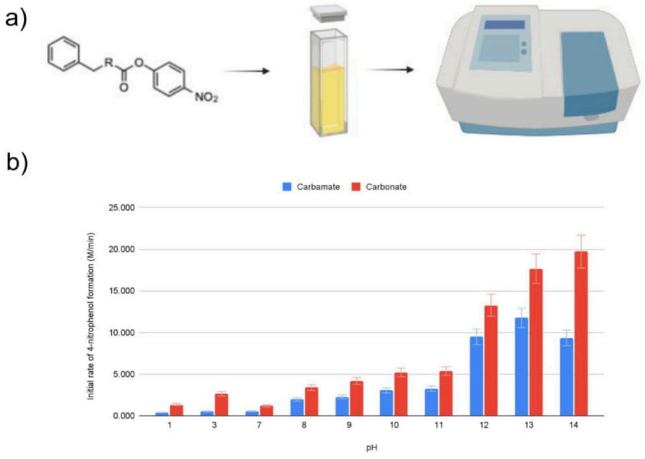

Figure 2: Kinetics of protecting group hydrolysis. A) Graphical representation of the workflow to obtain spectroscopic data. To prepare the cuvettes for UV-visible analysis, the 4-nitrophenol compounds were dissolved at a uniform concentration in a solution of DMSO/water at pH 1, 3, 7, 8, 9, 10, 11, 12, 13, and 14 (n=3). **B)** Relative initial rates of hydrolysis of the 4-nitrophenyl carbamate and carbonate were compared by plotting the final absorbance after 10 minutes of hydrolysis at 413 nm, which is near the maximum absorbance of 4-nitrophenol in solution. Error bars represent the standard error.

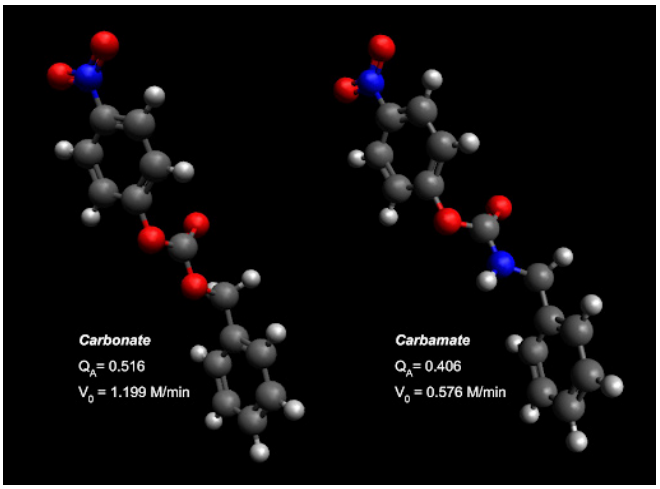

Figure 3: Mulliken charge calculations. Mulliken values of the carbonyl carbon of the carbonate-conjugated protecting group (left) and carbamate-conjugated protecting group (right) were determined by DFT calculations. QA represents the partial charge of the carbonyl carbon and V0 represents the initial rate of hydrolysis in pH 7 for each compound.

data, along with a Beer's Law plot of 4-nitrophenol, were then used to derive the initial rate of hydrolysis. Our results demonstrate that the deprotection was most effective at pH 12 and above, and minimal deprotection occurred in neutral and acidic pH (**Figure 2B**).

Furthermore, to rationalize how the two compounds displayed different rates of hydrolysis, we performed computational calculations to identify the local charge densities of each compound. Each substrate was optimized via density functional theory (DFT), a quantum mechanical method that uses an electron density functional to predict the energy of a system. Then, Mulliken charges at the carbonyl carbon of the geometry-optimized structures were extracted (QA of the carbonate = 0.516, QA of the carbamate = 0.406), representing local charge density (**Figure 3**). We calculated that 4-nitrophenyl benzylcarbonate would have a more positive Mulliken charge compared to 4-nitrophenyl benzylcarbamate, as the adjacent oxygen would withdraw more charge density than the adjacent nitrogen.

DISCUSSION

Using UV-vis spectrophotometry we recorded the rates of deprotection of the carbonate and carbamate in different pH environments, and using computational analysis we rationalized why the carbonate deprotected more rapidly than the carbamate in basic conditions. The trend observed in the kinetic studies involving UV-vis spectrophotometry was that under acidic and neutral conditions, minimal to no hydrolysis of either the carbamate or the carbonate occurred. However, in increasingly basic conditions, the initial rate of hydrolysis of the carbonate continuously increased, while the initial rate of hydrolysis of the carbamate increased towards a maximum at pH 13. Moreover, at all pH levels, the hydrolysis of the carbonate occurred more rapidly than that of the carbamate.

The difference in the rates of hydrolysis is attributed to the difference in local charge density at the carbonyl carbon of each compound, which was supported by the computational analyses. The DFT calculations revealed that the Mulliken value of the carbonyl carbon of the carbonate-conjugated protecting group was greater than that of the carbonyl carbon of the carbamate-conjugated protecting group, which indicated that the former is less electron dense. This is consistent with the greater electron-withdrawing capabilities of the adjacent oxygen atom on the central carbonate carbon compared to the adjacent amide on the central carbamate carbon (17). Since the mechanism of deprotection in acidic and basic conditions involves nucleophilic addition onto the carbonyl carbon, our DFT calculations supported our spectroscopic finding that the 4-nitrophenyl carbonate, which has a more electron-poor carbonyl carbon, hydrolyzed quicker in both acidic and basic conditions, as the nucleophilic addition is more favorable.

Our results demonstrate that 4-nitrophenol carbonates and carbamates may be widely adopted as base-labile protecting groups that are stable in acidic and neutral conditions and rapidly deprotect in basic environments of pH values higher than 12. Our proposed one-step synthesis schematic used commercially available and economical reagents and allowed for high purification yields between 72%-94%, ensuring that this protecting group strategy will preserve synthetic efficiency while allowing for selective deprotection of orthogonal groups. Furthermore, 4-nitrophenol allowed for easy reaction monitoring due to its bright yellow color when released in solution.

To investigate the applicability of the 4-nitrophenyl carbonate and carbamate protecting group, we plan to compare our strategy to those of other base-labile protecting groups, such as Fluorenylmethyloxycarbonyl, by probing for selective deprotection under different solvation conditions and by testing on more complex substrates than the ones investigated in this experiment. To this end, the laboratory synthesis of a multiply protected compound with different base-labile protecting groups will be conducted in the future. Additionally, although current deprotection steps often involve treatment with an acid or base, this is not a sustainable method in the large-scale synthesis of non-small molecules where the product itself may have other groups that are sensitive to solution pH (18-20). Thus, we will also investigate alternative deprotection strategies, such as treatment with an oxidizing or reducing agent.

MATERIALS AND METHODS

Protection of benzyl alcohol was achieved via acylation by 4-nitrophenyl chloroformate (**Figure 4**). Benzyl alcohol (1 eq., Sigma-Aldrich) was dissolved in methylene chloride, along with triethylamine (1 eq., AK Scientfic). Next, 4-nitrophenyl chloroformate (1.2 eq., AK Scientific) was added to the reaction mixture and the reaction was monitored to completion via thin-layer chromatography. The crude reaction mixture was concentrated in vacuo and purified on silica gel

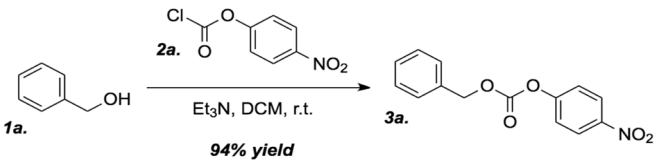

Figure 4: Chemical synthesis of 4-nitrophenyl benzylcarbonate

flash chromatography with a gradient of 0-30% ethyl acetate in hexanes, yielding off-white crystals of 4-nitrophenyl benzylcarbonate in 94% yield. Solvents used in purification were purchased from Stellar Chemical, JT Baker, or Fisher, and used without further purification.

Protection of benzylamine was achieved via acylation by 4-nitrophenyl chloroformate (**Figure 5**).

4-nitrophenyl chloroformate (1 eq., AK Scientific) was dissolved in methylene chloride and suspended over an ice bath, along with triethylamine (1 eq., AK Scientific). Next, benzylamine (1.8 eq., AK Scientific) was added dropwise to the reaction mixture, and the reaction was monitored to completion via TLC. Purification of this compound yielded off-white crystals in 72% yield and was achieved in the same manner as the 4-nitrophenyl benzylcarbonate.

Mulliken charge calculations

Each substrate was constructed virtually on Avogadro and optimized using DFT by ORCA, from which Mulliken charges at the carbonyl carbon were extracted (21, 22). In all DFT calculations, Conductor-like Polarizable Calculation Model (CPCM) implicit solvation with the dielectric constant of water was used as the solvation model, B3LYP was used as the functional, and def2-SVP was used as the basis set. We chose these settings because CPCM with the dielectric constant of water is a fair mimic of biological conditions, B3LYP is commonly used for moedling organic molecules, and def2-svp has a good balance of comparative computational cost and accuracy (23, 24).

Characterization of 3a and 3b

All compounds were characterized by 1H and 13C{1H} nuclear magnetic resonance (NMR) spectroscopy (Nanalysis NMReady 60 MHz NMR spectrometer) using deuterated chloroform (Cambridge Isotope Laboratories, >99.8% D, 0.1% tetramethylsilane (TMS)) as an internal standard, Fourier-transform infrared (FT-IR) spectroscopy (Thermo Scientific iS5 Nicolet FT-IR spectrometer, iD5 Attenuated Total Reflectance (ATR) assembly), and UV-visible spectroscopy

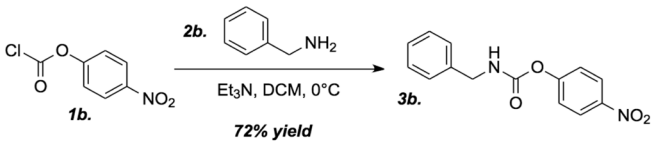

Figure 5: Chemical synthesis of 4-nitrophenyl benzylcarbamate

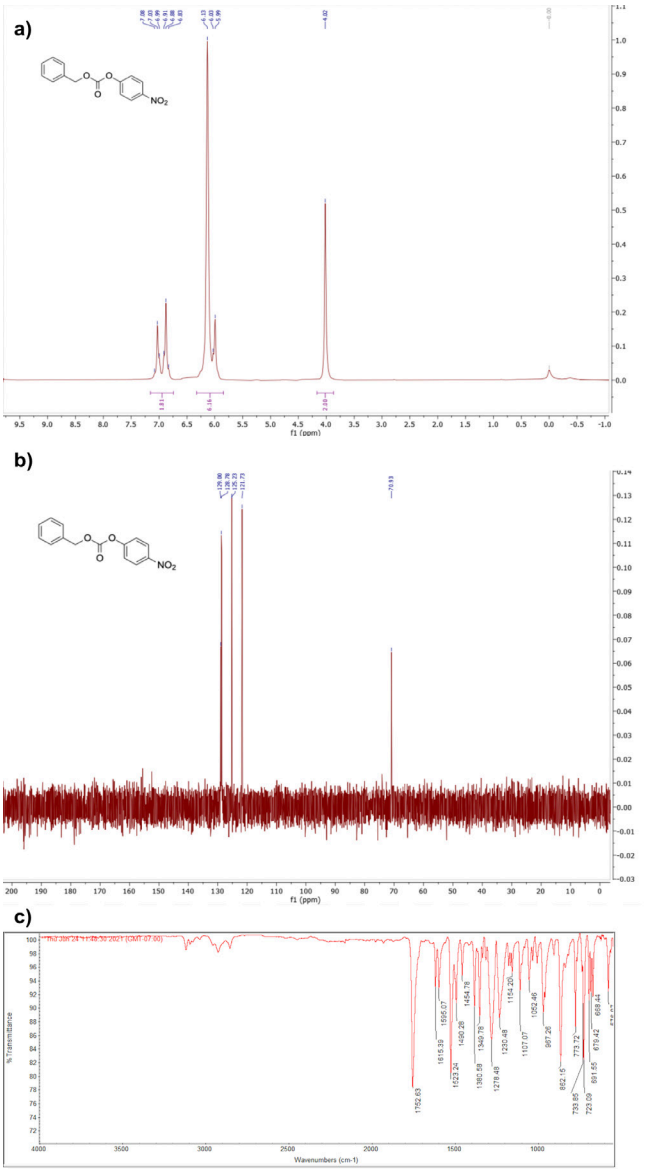

Figure 6: Spectroscopic characterization of 4-nitrophenyl benzylcarbonate (Compound 3a). A) 1H NMR (61 MHz, Chloroform-d) line listing. δ 7.08 – 6.83 (d, J = 9.2 Hz, 2H), 6.13 – 5.99 (m, 6H), 4.02 (s, 2H). **B)** 13C NMR (15 MHz, Chloroform-d) line listing. δ 129.00, 128.78, 125.23, 121.73, 70.93. **C)** FT-IR (neat, ATR) line listing. 1753, 1615, 1595, 1523, 1490, 1455, 1381, 1350, 1278, 1230, 1154, 1052, 967, 862, 774, 734, 723, 692, 679, 668 cm^{-1}.

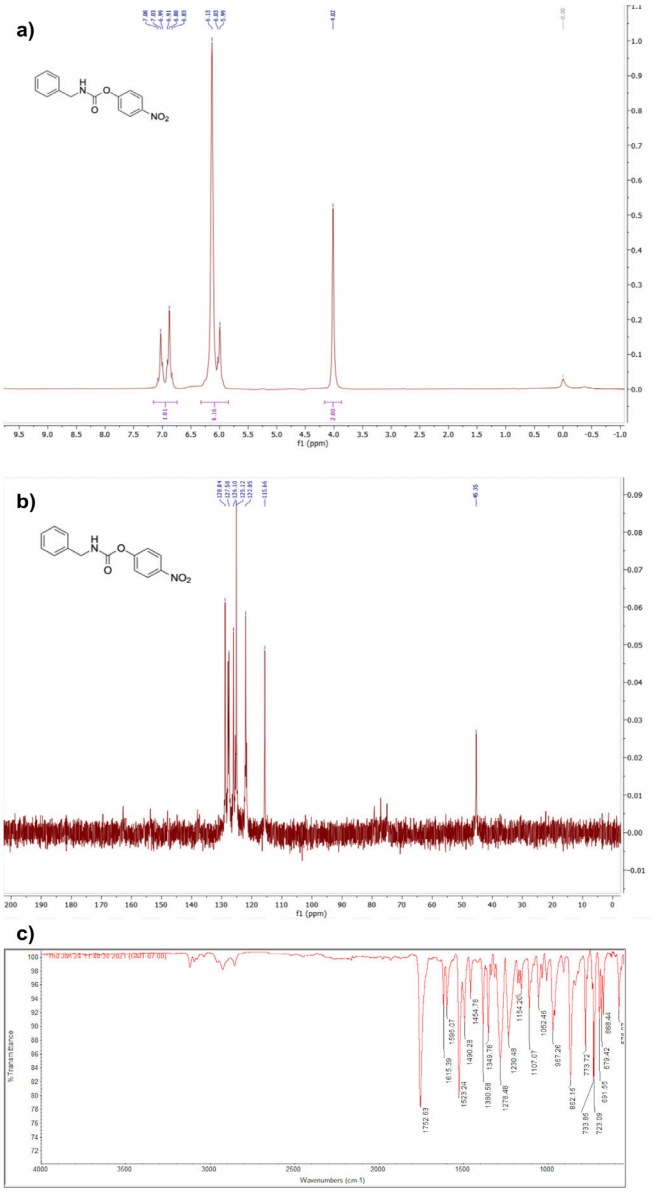

Figure 7: Spectroscopic characterization of 4-nitrophenyl benzylcarbamate (Compound 3b). A) 1H NMR (61 MHz, Chloroform-d) line listing. δ 8.42 – 8.05 (d, J = 9.2 Hz, 2H), 7.57 – 6.78 (m, 6H), 5.55 (br, s, 1H), 4.48 (d, J = 5.9 Hz, 2H). **B)** 13C NMR (15 MHz, Chloroform-d) line listing. δ 128.84, 127.58, 126.10, 125.12, 122.05, 115.66, 45.35. **C)** FT-IR (neat, ATR) line listing. 1753, 1615, 1595, 1523, 1490, 1455, 1381, 1350, 1278, 1230, 1154, 1052, 967, 862, 774, 734, 723, 692, 679, 668 cm^{-1}.

(BioRad SmartSpec 3000 UV-vis spectrophotometer, quartz cuvette) (**Figures 6-7**).

UV-visible spectroscopy

A Beer's Law plot of 4-nitrophenol was produced by collecting spectroscopic data at various micromolar concentrations using a Spectronic Genesys 5 UV-visible spectrophotometer. The

4-nitrophenol was prepared in 10% dimethyl sulfoxide (DMSO; DMSO Store, 99.995%) and 90% 10 mM Tris buffer at concentrations of 1.25 μM, 2.5 μM, 5 μM, and 10 μM. The peak absorbance of 4-nitrophenol was determined to be at 413 nm, which did not overlap with the absorption spectra of the benzylcarbonate and benzylcarbamate substrates.

pH solutions and compound solutions of 4-nitrophenyl benzylcarbonate and 4-nitrophenyl were prepared separately, then added into a glass cuvette together to monitor the rate of deprotection. 1 mM solutions of each of the two compounds were prepared in DMSO, and pH solutions were prepared with 1mM Tris base in deionized water. Basic solutions were prepared by increasing the concentration of Tris base, and

acidic solutions were prepared by adding hydrochloric acid. The acidity or basicity of each solution was then tested with litmus paper and adjusted accordingly. After 1800 µL of pH solution was added to 200 µL of compound solution in a glass cuvette, the absorbance at 413 nm was measured every minute for 10 minutes. All experiments were repeated in triplicate. The rate of deprotection of each substrate by each enzyme was determined by calculating the average rate of increase of the concentration of 4-nitrophenol over the first 10 minutes of hydrolysis.

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