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O−N Intramolecular Alkoxycarbonyl Migration of Typical Protective Groups in Hydroxyamino Acids

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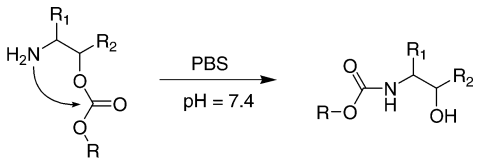

PBS = phosphate buffered saline

O−N Intramolecular alkoxycarbonyl (carbonate−carbamate) migration was found to occur as a common reaction of hydroxyamino acids under mild basic aqueous conditions with no formation of side products. Carbonate protective groups migrate to produce amino-protected carbamate derivatives of hydroxyamino acids with high efficiency and purity.

Introduction

O−N Intramolecular acyl migration is a well-known rearrangement reaction that has been applied in peptide ligation,[1] the synthesis of "difficult" peptides,[2−9] peptidomimetics,[10−15] and lactams,[16,17] as well as in water-soluble prodrug strategies[7,18−22] and the design of irreversible enzyme inhibitors.[23] This type of reaction is also observed in a variety of natural products,[24] and S−N intramolecular acyl migration has recently been extensively explored.[1,25] This suggests that the concept of the O−N intramolecular acyl migration can be effectively applied to similar rearrangement reactions. We focused on one such reaction, O−N intramolecular alkoxycarbonyl (carbonate−carbamate) migration. From an extensive literature search, it is assumed that the reports for this migration are rare and that migration occurred only under forcing conditions.[26] On the other hand, the partial hydrolysis of carbonate[20] or the side production of oxazolidinone[27] has been reported under milder reaction conditions. Herein, we demonstrated that carbonate protective groups can undergo an O−N intramolecular alkoxycarbonyl migration reaction to produce amino-protected carbamate derivatives. Therefore, this atom-economical reaction can provide a useful tool for organic chemists. For example, it can be used to shorten a synthesis of some target molecule in a similar manner as the acyl migration reaction did.[10−15]

Recently, in a study on "chemical pharmaceutics", we reported the first successful application of O−N intramolecular alkoxycarbonyl migration[28] in the development of water-soluble taxoid prodrugs (isotaxoids),[29] which are *O*-alkoxycarbonyl isoforms at a characteristic α-hydroxy-*β*-amino acid (phenylisoserine) moiety, and released parent anti-cancer agents with no side-product formation under physiological conditions.[29]

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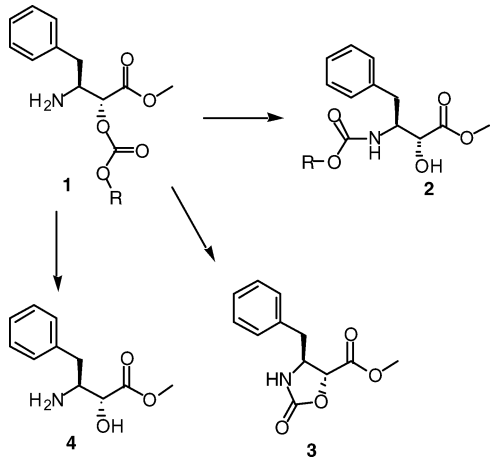
SCHEME 1. Possible Types of Carbonate Conversion in α-Hydroxy-β-amino Acid Residue under Mild Basic Conditions

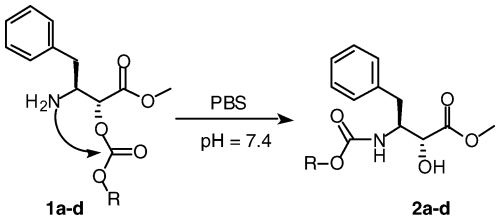


Hence, this atom-economical reaction proceeding in aqueous solvent systems is also recognized as a green-chemistry reaction. However, the application of this reaction in synthetic organic chemistry under mild basic conditions has not yet been investigated. α-Hydroxy-β-amino acid derivatives were selected for this study on the basis of our previous observation in the prodrug study.

Results and Discussion

An α-hydroxy-β-amino acid, phenylnorstatine (Pns, (*2R*,*3S*)-3-amino-2-hydroxy-4-phenylbutanoic acid), is a well-known hydroxymethylcarbonyl isostere used for a variety of potent peptidomimetic inhibitors for biologically important enzymes.[30] We selected this amino acid to understand the possible reactions occurring in *O*-alkoxycarbonyl compounds under mild basic aqueous conditions (pH 7.4): (a) O−N intramolecular alkoxycarbonyl migration (**2**),[20,27,29] (b) oxazolidinone formation (**3**),[27,31] and (c) simple hydrolysis (**4**;[20] Scheme 1). Subsequently, we synthesized compounds **1a**−**d** (see Experimental Section and Supporting Information). These model compounds have typical alkoxycarbonyl residues, that is, ethyloxycarbonyl, 9-fluorenylmethoxycarbonyl (Fmoc), 2,2,2-trichloroethoxycarbonyl (Troc), and benzyloxycarbonyl (Z) groups, which are widely used for the protection of both hydroxyl and amine groups (Table 1). All expected products, **2a**−**d**, **3**, and **4**, were also synthesized independently by standard methods (see Experimental Section and Supporting Information). Compounds **1a**−**d** were dissolved in phosphate buffered saline (PBS, pH 7.4) and stirred at room temperature, and the reaction was monitored and analyzed by HPLC. In all cases, **1a**−**d** were completely consumed, and the quantitative formation of carbamates **2a**−**d** was observed (Table 1 and Figure 1S from Supporting Information). Neither hydrolysis nor formation of side products including oxazolidinone was detected. The formation of **2a**−**d** was also confirmed by mass spectroscopy. The result with no hydrolysis of the Fmoc group in **1b** was quite surprising considering its instability under basic conditions.[32] However, this is consistent with our previous assumption that carbonate hydrolysis under mild basic conditions is limited only to the Boc group or its related structures.[29]

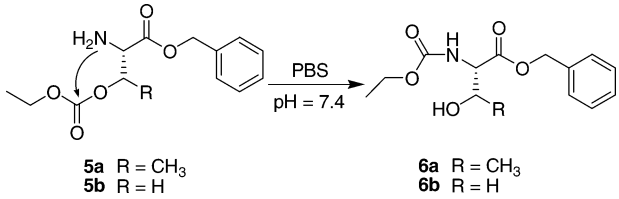
TABLE 1. O−N Intramolecular Alkoxycarbonyl Migration in Pns Derivatives



substrate	product	R	time (min)	yield[a] (%)
1a	**2a**	C_2H_5	60	99
1b	**2b**	9-fluorenylmethyl	960	99
1c	**2c**	CH_2CCl_3	5	99
1d	**2d**	$CH_2C_6H_5$	60	99

[a] The yield established by HPLC was quantitative, as one pure product was detected.

SCHEME 2. O−N Intramolecular Alkoxycarbonyl Migration in Natural Amino Acid Derivatives



The isolation yield of the desired product (**2a**) was also measured according to the procedure shown in Experimental Section. As expected, compound **3** or **4** was not detected, and carbamate **2a** was isolated as a pure compound with 99% yield.

To understand why the known oxazolidinone formation was not observed, carbonates **1a**−**d** were treated with K_2CO_3 in methanol, according to a reported procedure.[31] However, the formation was not detected, and carbamates **2a**−**d** were obtained as the exclusive products. Therefore, two natural amino acid carbonate derivatives, **5a** and **5b**, were synthesized (see Experimental Section and Supporting Information) and treated under the same aqueous condition described for compound **1a** (Scheme 2). Even in these cases, O−N intramolecular alkoxycarbonyl migration predominantly formed products **6a** and **6b** with isolated yields of 98 and 89%, respectively, and oxazolidinone formation or carbonate hydrolysis was not detected. The lower yield of compound **6b** was due to the hydrolysis of the unstable benzyl ester under mild basic aqueous conditions. However, hydrolysis of the benzyl ester was not connected with the O−N intramolecular alkoxycarbonyl migration reaction. This suggests that O−N intramolecular alkoxycarbonyl migration is a common and exclusive reaction for *O*-acyl-α-hydroxy-β-amino acids and β-hydroxy-α-amino acids. Thus, our findings in O−N

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intramolecular migration of common protective groups provide a highly promising procedure for peptide chemists, also in the context of green chemistry. This reaction can also be directly applied to carbohydrate chemistry, because the migration of protective groups is an important issue in the regioselective modifications of sugars.[33]

In conclusion, we demonstrated herein that O−N intramolecular alkoxycarbonyl (carbonate−carbamate) migration commonly proceeded under pure aqueous mild basic conditions (pH 7.4). In particular, carbonate groups can migrate to produce carbamate derivatives of hydroxyamino acids with high efficiency and purity, without byproduct formation (hydrolysis of the ester bond is not a byproduct of this migration). This finding offers a useful tool for organic chemists, as typical and widely used protective groups can undergo this reaction.

Experimental Section

(*2R,3S*)-3-Amino-2-[(ethoxycarbonyl)oxy]-4-phenylbutanoic Acid Methyl Ester Hydrochloride, 1a. Ethoxycarbonyl chloride (10 μL, 103 μmol) was added to a stirring solution of (*2R,3S*)-2-hydroxy-3-[(*tert*-butoxycarbonyl)amino]-4-phenylbutanoic acid methyl ester[34] (16.0 mg, 51.6 μmol) in dry CH_2Cl_2 (1 mL) and dry pyridine (1 mL), and the mixture was stirred under an argon atmosphere at room temperature for 1 h. The reaction mixture was then diluted with AcOEt and successively washed with water, 1M hydrochloric acid (two times), and brine. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The resulting oil was dissolved in 4 M HCl in dioxane (1 mL) with anisole (11 μL, 103 μmol), and the reaction mixture was stirred for 30 min at room temperature. The organic solvent was evaporated under reduced pressure, and the reaction mixture was directly applied to preparative HPLC, which was eluted with a linear gradient of 10−40% CH_3CN in 12 mM aqueous HCl over 60 min at a flow rate of 5 mL/min. The desired fraction was collected and lyophilized to give a white powder of **1a** as an HCl salt (12.0 mg, 37.8 μmol, 73%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.41−7.27 (m, 5H, CH), 4.89 (d, $^3J_{(H,H)}$ = 2.5 Hz, 1H, CH), 4.29 (q, $^3J_{(H,H)}$ = 7.1 Hz, 2H, CH_2), 4.08 (ddd, $^3J_{(H,H)}$ = 2.5, 6.7, 9.0 Hz, 1H, CH), 3.79 (s, 3H, CH_3), 3.08, 3.01 (2 dd, $^2J_{(H,H)}$ = 13.8 Hz, $^3J_{(H,H)}$ = 6.7, 9.0 Hz, 2H, CH_2), 1.34 (t, $^3J_{(H,H)}$ = 7.1 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD, 100 MHz): δ_c 168.8, 155.1, 135.7, 130.43, 130.38, 129.0, 73.4, 66.6, 54.0, 53.8, 36.7, 14.5. HRMS (FAB^+) calcd for $C_{14}H_{20}NO_5$ [M^+ + H], 282.1341; found, 282.1347. Purity was higher than 99% (HPLC analysis at 230 nm).

(*2R,3S*)-3-[(Ethoxycarbonyl)amino]-2-hydroxy-4-phenylbutanoic Acid Methyl Ester, 2a.[35] (*2R,3S*)-3-Amino-2-hydroxy-4-phenylbutanoic acid methyl ester hydrochloric acid salt, **4**·HCl (20.0 mg, 81.4 μmol), was dissolved in CH_2Cl_2 (0.5 mL), and a saturated solution of $NaHCO_3$ (0.5 mL) was added. Ethoxycarbonyl chloride (9.7 μL, 102 μmol) was then added with vigorous stirring. After 1 h, the next portion of ethoxycarbonyl chloride (9.7 μL, 102 μmol) was added, and after an hour, the reaction mixture was diluted with diethyl ether and washed with water, $NaHCO_3$, water, 1 M hydrochloric acid, water, and brine. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure to give **2a** (22 mg, 78.2 μmol, 96%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.30−7.18 (m, 5H, CH), 4.20−4.16 (m, 1H, CH), 4.09 (d, $^3J_{(H,H)}$ = 2.4 Hz, 1H, CH), 4.00−3.94 (m, 2H, CH_2), 3.67 (s, 3H, CH_3), 2.92, 2.81 (2 dd, $^2J_{(H,H)}$ = 13.6 Hz, $^3J_{(H,H)}$ = 7.8, 7.9 Hz, 2H, CH_2), 1.16 (t, $^3J_{(H,H)}$ = 7.1 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD, 100 MHz): δ_c 175.1, 158.5, 139.5, 130.4, 129.5, 127.6, 72.0, 61.9, 56.7, 52.6, 38.8, 14.9. HRMS (FAB^+) calcd for $C_{14}H_{20}NO_5$ [M^+ + H], 282.1341; found, 282.1335. Purity was higher than 99% (HPLC analysis at 230 nm).

(*4S,5R*)-4-Benzyl-5-[(methoxy)carbonyl]-1,3-oxazolidin-2-one, 3.[36] (*2R,3S*)-3-Amino-2-hydroxy-4-phenylbutanoic acid methyl ester hydrochloric acid salt, **4**·HCl (75.0 mg, 305 μmol), was dissolved in anhydrous THF (3 mL), and Et_3N (51 μL, 366 μmol) was added followed by 1,1′-carbonyldiimidazole (74 mg, 475 μmol) at 0 °C.[37] The cloudy reaction mixture was stirred overnight at room temperature, diluted with AcOEt, and washed consecutively with 10% citric acid, $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was applied to preparative HPLC, which was eluted with a linear gradient of 20−60% CH_3CN in 0.1% aqueous TFA over 40 min at a flow rate of 5 mL/min. The desired fraction was collected and lyophilized to give **3** (32.7 mg, 139 μmol, 46%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38−7.20 (m, 5H, CH), 5.58 (br s, 1H, NH), 4.72 (d, $^3J_{(H,H)}$ = 4.6 Hz, 1H, CH), 4.15−4.11 (m, 1H, CH), 3.81 (s, 3H, CH_3), 3.07, 2.90 (2dd, $^2J_{(H,H)}$ = 13.6 Hz, $^3J_{(H,H)}$ = 5.0, 8.5 Hz, 2H, CH_2), 2.47 (br s, 1H, OH). HRMS (FAB^+) calcd for $C_{12}H_{14}NO_4$ [M^+ + H], 236.0923; found, 236.0928.

(*2R,3S*)-3-Amino-2-hydroxy-4-phenylbutanoic Acid Methyl Ester Hydrochloride, 4.[38,39] (*2R,3S*)-2-Hydroxy-3-[(*tert*-butoxycarbonyl)amino]-4-phenylbutanoic acid methyl ester[34] (1.0 g, 3.23 mmol) was dissolved in 4 M HCl in dioxane (16 mL) with anisole (0.7 mL, 6.46 mmol), and the reaction mixture was stirred for 1 h at room temperature. The organic solvent was evaporated, and the solid was washed with diethyl ether to give a white powder of **4** as a HCl salt (0.776 g, 3.16 mmol, 98%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.39−7.28 (m, 5H, CH), 4.12 (d, $^3J_{(H,H)}$ = 2.5 Hz, 1H, CH), 3.82 (ddd, $^3J_{(H,H)}$ = 2.5, 6.4, 9.0 Hz, 2H, CH_2), 3.74 (s, 3H, CH_3), 3.07, 3.00 (2 dd, $^2J_{(H,H)}$ = 13.6 Hz, $^3J_{(H,H)}$ = 6.6, 9.0 Hz, 2H, CH_2). HRMS (FAB^+) calcd for $C_{11}H_{16}NO_3$ [M^+ + H], 210.1130; found, 210.1135.

***O*-Ethoxycarbonyl-L-threonine Benzyl Ester Hydrochloride, 5a.** Ethoxycarbonyl chloride (17.8 μL, 186 μmol) was added to the stirring solution of *N*-(*tert*-butoxycarbonyl)-L-threonine benzyl ester[40] (28.8 mg, 93.2 μmol) in dry $CHCl_3$ (1 mL) and dry pyridine (1 mL), and the mixture was stirred under an argon atmosphere at room temperature for 1 h. Another portion of ethoxycarbonyl chloride (17.8 μL, 93.2 μmol) was added, and after two more hours, the reaction mixture was diluted with AcOEt and successively washed with water, 1 M hydrochloric acid (two times), and brine. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The resulting oil was dissolved in 4 M HCl in dioxane (1.5 mL) with anisole (20 μL, 187 μmol), and the reaction mixture was stirred for 1 h at room temperature. The organic solvent was evaporated under reduced pressure, and the reaction mixture was directly applied to preparative HPLC, which was eluted with a linear gradient of 10−40% CH_3CN in 12 mM aqueous HCl over 60 min at a flow rate of 5 mL/min. The

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(34) The compound was purchased from Nippon Kayaku Co., Ltd. (Tokyo, Japan).

(35) Compounds **2**−**4** were synthesized as standards for monitoring the O−N intramolecular alkoxycarbonyl migration reaction in compounds **1a**−**d**.

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desired fraction was collected and lyophilized to give a white powder of **5a** as a HCl salt (22.4 mg, 70.5 μmol, 76%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.42−7.33 (m, 5H, CH), 5.32 (dq, $^3J_{(H,H)}$ = 3.5, 6.6 Hz, 1H, CH), 5.34, 5.23 (2d, $^2J_{(H,H)}$ = 12.0 Hz, 2H, CH_2), 4.37 (d, $^3J_{(H,H)}$ = 3.3 Hz, 1H, CH), 4.14, 4.09 (2dq, $^2J_{(H,H)}$ = 10.6 Hz, $^3J_{(H,H)}$ = 7.1 Hz, 2H, CH_2), 1.42 (d, $^3J_{(H,H)}$ = 6.8 Hz, 3H, CH_3), 1.24 (t, $^3J_{(H,H)}$ = 7.1 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD, 100 MHz): δ_c 168.1, 155.0, 136.2, 129.92, 129.88, 129.7, 72.7, 69.6, 65.8, 57.6, 16.9, 14.5. HRMS (FAB$^+$) calcd for $C_{14}H_{20}NO_5$ [M$^+$ + H], 282.1341; found, 282.1346. Purity was higher than 99% (HPLC analysis at 230 nm).

Standard Procedure for HPLC Monitoring of O−N Alkoxycarbonyl Migration. (*2R*,*3S*)-3-[(Ethoxycarbonyl)amino]-2-hydroxy-4-phenylbutanoic Acid Methyl Ester, 2a. Compound **1a** was dissolved in PBS (pH 7.4) to give a 0.1 mM solution and stirred at room temperature, and the reaction was monitored and analyzed by HPLC in comparison with synthetic **2a**, **3**, and **4**. The production of only **2a** was observed.

Standard Procedure for the Isolation of O−N Alkoxycarbonyl Migration Reaction Product. (*2R*,*3S*)-3-[(Ethoxycarbonyl)-amino]-2-hydroxy-4-phenylbutanoic Acid Methyl Ester, 2a. Namely, **1a** (11.7 mg) was suspended in 3.7 mL of PBS (pH 7.4) to give a 10 mM solution (this concentration was establish to prevent a pH change during the process) and vigorously stirred at 25 °C overnight. The product, **2a**, was then extracted with AcOEt. Carbamate **2a** was isolated as a pure compound with 99% yield (10.3 mg). The spectroscopic data of **2a** synthesized by this method and by the standard method were identical.

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Supporting Information Available: Characterization data for compounds **1b**−**d**, **2b**−**d**, **5b**, and **6a**,**b** and copies of ^{1}H and ^{13}C NMR spectra and HPLC profiles for new compounds **1a**−**d**, **2a**−**d**, **5a**,**b** and **6a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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