Homocarbonates as Substrates for the Enantioselective Enzymatic Protection of Amines

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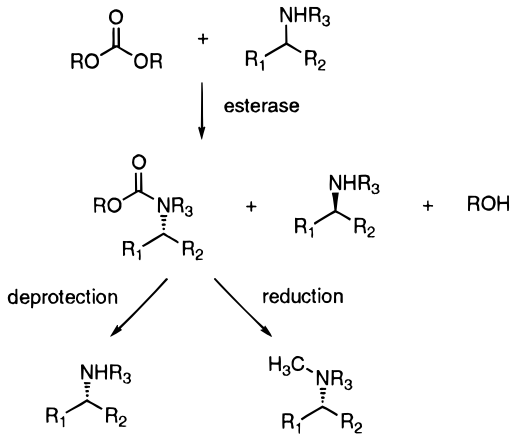
The irreversible enzymatic acylation of alcohols in high concentrations of organic solvents using lipases or serine proteases as catalysts and activated esters such as choloroethyl, trifluoroethyl, cyanomethyl, enol, oxime, or thio esters or anhydrides as acylating reagents has been shown to be useful in organic synthesis.[1] Of these reagents, enol esters are the most widely used,[1,2] as the rate of enzymatic acylation is relatively fast and the released enol is spontaneously tautomerized to a ketone, making the process irreversible and free of inhibition caused by the leaving alcohol and the product easy to isolate. These acylating reagents, however, cannot be used in the enzymatic acylation of amines, as they are too reactive and give high background reactions. Recently, the use of vinyl carbonates to protect amines using the lipase from *Candida antarctica* has been reported.[3] These unsymmetrical carbonates are, however, not readily available and may be too reactive to avoid nonenzymatic reactions.

We report here a novel enzymatic method for protecting amines as carbamates with high enantioselectivity using commercially available and low-cost homocarbonates as substrates for lipases and proteases (Scheme 1).[4] The reactions are irreversible, as the products carbamates are not substrates for the serine-type esterases. Furthermore, the symmetrical structure of the homocarbonates gives unambiguously a single carbamate product, making the process very simple. The carbamate can be easily deprotected or converted to the N-methyl derivative by reaction with $LiAlH_4$, providing a new procedure for the chemoenzymatic methylation of amines.

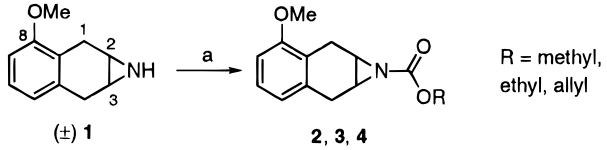
In a preliminary experiment, racemic aziridine **1** was stirred at ambient temperature with diallyl carbonate in phosphate buffer (pH 8.0) containing subtilisin BPN′.[5] Carbamate **4** was obtained in 50% yield after 2 h. Both dimethyl and diethyl carbonates reacted more slowly; after 90 h, **2** and **3** were obtained in 15 and 24% yield, respectively.[6] Disappointingly, the enantiomeric excess (ee) of each product, determined by HPLC using a chiral column (Chiralcel OD-H, Daicel), was <25%. Adding 75% 1,4-dioxane to the diallyl carbonate system gave a maximum yield of 39% after 74 h and 54% ee.[6] Switching from subtilisin BPN′ to *Candida cylindracea* lipase (CCL, also called *Candida rugosa* lipase, CRL) and using the carbonates as solvents at room temperature for 45 h gave better results (Scheme 2).[5,6] Due to its crystalline nature, dibenzyl carbonate could not be used in this procedure. Racemic **4** was also prepared by a standard procedure and used as a reference for spectroscopic and chromatographic analyses.[7]

When primary amines were mixed with diallyl carbonate as solvent, a significant background reaction was observed without enzyme. In aqueous buffer, however, no background reaction was detected, and the use of subtilisin BPN′ appeared to be useful for the enzymatic protection of amines under these conditions. To investigate the chemo- and enantioselectivity of this process, we chose three multifunctional substrates that could undergo different enzymatic reactions. When racemic **5** was used as substrate, the reaction was carried out in phosphate buffer (pH 8.0) with subtilisin BPN′ and diallyl carbonate (Scheme 3). After 69 h, **6** was obtained in 49% yield and 78% ee, as determined by HPLC (Chiralpak AD, Daicel), based on the *O*-(*p*-anisoyl) derivative **7** (Scheme 3).[6] Carbamate **6** was subsequently deprotected according to a standard procedure to provide **5** after enzymatic resolution.[8] The optical rotation of the obtained **5** ($[\alpha]_D$ +14°, *c* 0.8, H_2O) was then compared with the published value to establish the absolute configuration

Scheme 1



Scheme 2[a]



(±) **1** **2**, **3**, **4**

product	R	yield [%]	ee [%]	$[\alpha]_D$ ($CHCl_3$)
2	methyl	24	27	-18° (c: 0.2)
3	ethyl	31	31	-14° (c: 0.1)
4	allyl	49	84	-15° (c: 0.9)

[a] Conditions: (a) Homocarbonate (1 mL), CCl (20 mg), room temperature, 45 h (0.1 mmol of **1** per milliliter of homocarbonate was used).

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(1) For a comprehensive documentation of enzymatic acylations, see: Klibanov, A.M. *Acc. Chem. Res.* **1990**, *23*, 114. Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994; p 72. Fang, J.-M.; Wong, C.-H. *Synlett* **1994**, *6*, 393.

(2) Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 6421. Degueil-Castaing, M.; Jeso, B. D.; Drouillard, S.; Maillard, B. *Tetrahedron Lett.* **1987**, *28*, 953. Wang, Y. F.; Lalonde, J. J.; Homongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200. Laumen, K.; Brietgoff, D.; Schneider, M.-P. *J. Chem. Soc., Chem. Commun.* **1988**, 1459.

(3) Pozo, M.; Pulido, R.; Gotor, V. *Tetrahedron* **1992**, *48*, 6477. Pozo, M.; Gotor, V. *Tetrahedron* **1993**, *49*, 10725. For other enzymatic resolutions of amines via reactions with esters to form amides, see: Kitaguchi, H.; Fitzpatrick, P. A.; Huber, J. E.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 3094. Fernandez, S.; Brieva, R.; Rebolledo, F.; Gotor, V. *J. Chem. Soc., Chem. Commun.* **1992**, 2885. Gotor, V.; Menendez, E.; Mouloungui, Z.; Gasset, A. *J. Chem. Soc., Chem. Commun.* **1993**, 2453.

(4) Dimethyl , diethyl, and diallyl carbonates were purchased from Aldrich Chemical Co. Dibenzyl carbonate was from Lancaster Synthesis Inc.

(5) Subtilisin BPN′ (Nagarse, type XXVII, 8.3 units/mg, P4789) and lipase from *Candida cylindracea* (CCL, type VII, 860 units/mg, L1754, EC 3.1.1.3) were purchased from Sigma.

(6) An identical reaction without enzyme did not give any detectable product over the same period.

(7) Sennyey, G.; Barcelo, G.; Senet, J.-P. *Tetrahedron Lett.* **1987**, *28*, 5809.

(8) Kunz, H.; Unverzagt, C. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 436.

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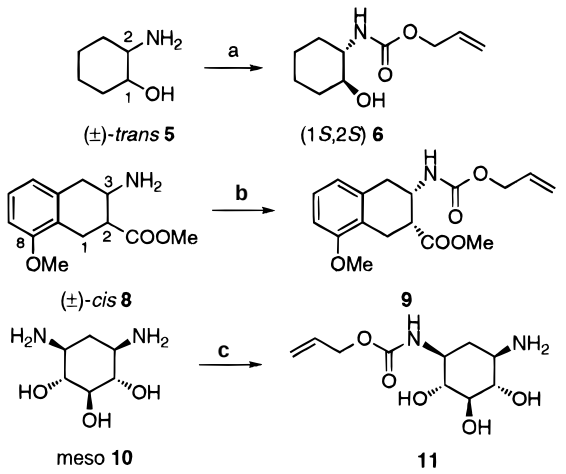
Scheme 3[a]

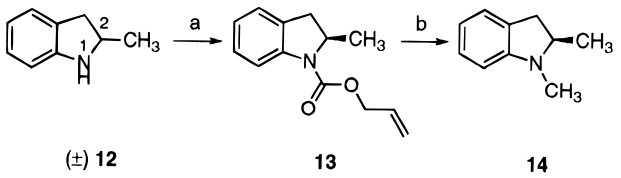


[a] Conditions: (a) Diallyl carbonate (0.6 mmol, 3 equiv), phosphate buffer (0.1 M, pH 8.0, 1 mL), BPN′ (10 mg), room temperature, 69 h, 49% yield, 78% ee. (b) Diallyl carbonate (0.2 mmol, 3.3 equiv), phosphate buffer (0.1 M, pH 8.0, 1 mL), BPN′ (6 mg), room temperature, 85 h, 45% yield, 93% ee. (c) Diallyl carbonate (1.23 mmol, 4 equiv × 4), HEPES buffer (0.2 M HEPES, 0.02 M $CaCl_2$, pH 7.8, 3 mL), DMF (3 mL), BPN′ (10 mg), room temperature, 1 week, 76% yield, >99% ee.

(1*S*,2*S*) for the enzyme product.[9] When dibenzyl carbonate was used instead of diallyl carbonate, only a trace of product was observed. This carbonate remained present in the reaction mixture, indicating that it is a poor substrate for the enzyme. Racemic β-amino ester **8** was treated similarly for 85 h, and carbamate **9** was obtained with 93% ee, as determined by HPLC (Chiralpak AD, Daicel), and in 45% yield without hydrolysis of the ester (Scheme 3).[6] When the concentration of **8** was increased to 0.1 M in the presence of 11 mg/mL subtilisin, **9** was obtained in 40% yield. At 0.3 M concentration of the substrate, **9** was obtained in 35% yield with use of 31 mg/mL of subtilisin. The meso substrate 2-deoxystreptamine (**10**) was converted to **11** through the use of subtilisin BPN′ and diallyl carbonate in HEPES buffer (200 mM, pH 7.8, 20 mM $CaCl_2$, 50% DMF). The chemo- and enantioselectivity of the reaction were complete despite five possible sites of reaction. The product was obtained in 76% yield and >99% ee, as determined by HPLC (Chiralpak AD, Daicel), based on the peracetylated *p*-toluenesulfonamide derivative **11a** (Scheme 3).[6]

(9) Faber, K.; Hönig, H.; Seufer-Wasserthal, P. *Tetrahedron Lett.* **1988**, *29*, 1903.

Scheme 4[a]



[a] (a) Diallyl carbonate (1.5 mmol, 1.5 equiv), phosphate buffer (0.1 M, pH 8.0, 10 mL), BPN′ (50 mg), room temperature, 92 h, 6% yield, 93% ee. (b) $LiAlH_4$ (8.7 equiv), ether, 0 °C, 2 h, 93%.

The process was also applied to the resolution of racemic secondary amine **12**. The enzymatic reaction was carried out for 92 h to yield **13** with 93% ee, as determined by HPLC (Chiralcel OD-H, Daicel), and in 6% yield (Scheme 4).[6] The low yield was attributed to the steric hindrance and unfavorable electronic effects occurring at the reacting center. The reaction was also attempted using diallyl carbonate as solvent and CCL as catalyst, but no reaction was observed. The carbamate was subsequently reduced to the N-methyl derivative using $LiAlH_4$ in ether to afford **14** in 93% yield. Resolution of racemic amino acids can also be carried out similarly with diallyl carbonate. Both serine and alanine, for example, were converted to the carbamates in ∼45% yield and >95% ee.

In summary, this report describes a novel enzymatic process using commercially available, low-cost homocarbonates for the protection of amines with high chemo- and enantioselectivity. Diallyl carbonate was shown to be the most useful substrate for the reaction, providing the highest yield and enantioselectivity. This versatile process can be carried out in aqueous (for primary and secondary amines) or organic solution (for secondary amines). Further studies are in progress to scale up some of these reactions and to find new substrates.

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Supporting Information Available: Experimental details for the preparation of **2**, **3**, **4**, **6**, **7**, **9**, **11**, **11a**, **13**, and **14** and ^1H and ^{13}C NMR spectra (11 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of this journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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