COMMUNICATION

ChemComm
www.rsc.org/chemcomm

β-Replacement reaction of serine-*O*-carbonate derivatives with thiols catalyzed by a pyridoxal model having an ionophore side-chain

Kazuyuki Miyashita, Hidenobu Murafuji, Hiroshi Iwaki, Eito Yoshioka and Takeshi Imanishi*
Graduate School of Pharmaceutical Sciences, Osaka University, 1–6 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: imanishi@phs.osaka-u-ac.jp

Received (in Cambridge, UK) 25th June 2002, Accepted 17th July 2002
First published as an Advance Article on the web 30th July 2002

Serine-*O*-carbonate derivatives, including peptides having a serine-*O*-carbonate residue at the N-terminal position, are catalytically transformed into *S*-substituted cysteine derivatives employing the pyridoxal model having an ionophore function in the presence of Li^+; this is the first artificial model mimicking cystathionine β-synthase.

β-Replacement reaction of the serine hydroxy group with a nucleophile, which is catalyzed by a pyridoxal-dependent enzyme, is an important reaction for biosynthesis of amino acids in biological systems (Fig. 1). Cystathionine β-synthase (CBS) and tryptophan synthase are typical examples of such biological reactions, in which the nucleophiles are homocysteine and tryptophan, respectively. From the viewpoint of synthetic organic chemistry, this reaction appears to be useful for the synthesis of various β-modified amino acids. Although some examples mimicking tryptophan synthase have been reported,[1,2] there is no example mimicking CBS. CBS is a biologically very important enzyme, which catalyzes β-replacement reaction of a serine hydroxy group with the thiol group of homocysteine to produce cystathionine.[3] Deficiency of this enzyme is known to cause homocystinuria.[4] Here we describe biomimetic transformation of serine derivatives into *S*-substituted cysteine derivatives employing pyridoxal models **1a** and **1b** (Fig. 2).

In previous papers, we have reported pyridoxal model compound **1a** having an ethoxyethoxy group at the C-3 position, a characteristic feature of which is that the aldimine is specifically activated by Li^+.[5] At first, we examined the reaction of aldimine **4a** prepared from the pyridoxal derivative **1a** and serine benzyl ester **3a** using thiophenol in the presence of lithium perchlorate. However, no reaction took place, and the starting aldimine **4a** was recovered. In order to increase the eliminating ability of the hydroxy group, we next employed aldimine **4b** which was obtained from serine *O*-benzoate or *O*-acetate **3b** and **1a**. In this case, **4b** disappeared quickly, but the desired product **5** was not obtained, and only a complex mixture was formed. As this appears to be due to the acid formed by the elimination, we eventually employed serine-*O*-carbonate **3c**, which forms carbon dioxide and methanol as the elimination products. The reaction of **4c** was found to smoothly take place as expected, to give the aldimine **5** in almost quantitative yield. However, acidic hydrolysis of the aldimine **5** resulted in only poor yield (*ca.* 10%) (Scheme 1).

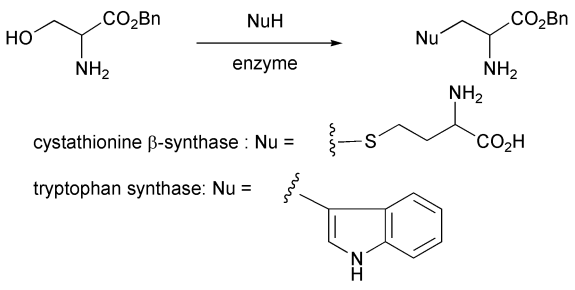


Fig. 1 β-Replacement reaction of Ser.

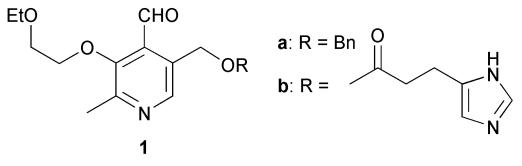
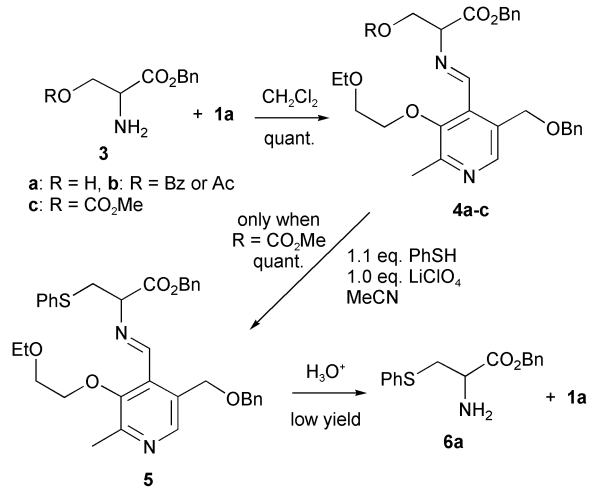


Fig. 2 Structures of pyridoxal model compounds.



Scheme 1 Stoichiometric β-replacement reaction.

Expecting an imine-exchange reaction between the aldimine **5** and serine-*O*-carbonate **3c**, we examined the reaction employing a catalytic amount of **1a** as shown in Fig. 3, and the results are summarized in Table 1.† The reaction proceeded in the presence of only 1% mol of **1a** (run 1). In this case, the yield based on the starting serine-*O*-carbonate **3c** was 76% and that based on **1a** was 7600%, which means that one molecule of **1a** can convert *ca.* 76 molecules of **3c** into the cysteine derivative **6a**. The reaction rate was improved by increasing the amount of **1a** (runs 2 and 3). However, the best yield based on **3c** was obtained when 5% mol of **1a** was employed (run 2). Regardless of the substituents on the benzene ring, other aromatic thiols also reacted, giving rise to *S*-aryl cysteines **6b–d** (runs 4–6). However, the reactions with alkanethiols required longer reaction time to complete, and the yields were lower than those with aromatic thiols (runs 7–10). We next examined the reaction with the pyridoxal model **1b** having an imidazole moiety,‡ expecting activation of the elimination of the *O*-carbonate moiety and/or activation of the nucleophile. Consequently, the reactions with alkanethiols proceeded smoothly, and the yields were improved (runs 7–10 *vs.* runs 11–14).

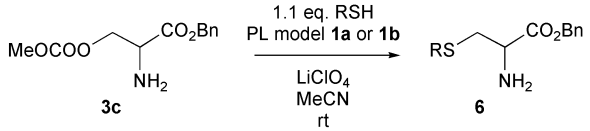


Fig. 3 Catalytic β-replacement reaction of **3c** with thiols.

DOI: 10.1039/b206061b

Table 1 Catalytic β-replacement reaction of **3c** to *S*-substituted cysteines **6**[a]

Run	PL (mol%)	$LiClO_4$ (mol%)	RSH	Time/h	Product	Yield based on **3c**	Yield based on **1**
1	**1a** (1)	1	Ph	38	**6a**	76	7600
2	**1a** (5)	5	Ph	5	**6a**	93	1860
3	**1a** (10)	10	Ph	2	**6a**	87	870
4	**1a** (5)	5	4-MeC_6H_4	5	**6b**	86	1720
5	**1a** (5)	5	4-$MeOC_6H_4$	5	**6c**	90	1800
6	**1a** (5)	5	4-$O_2NC_6H_4$	3	**6d**	92	1840
7	**1a** (5)	5	Bn	19	**6e**	61	1220
8	**1a** (5)	5	Et	22	**6f**	41	820
9	**1a** (5)	5	$HO(CH_2)_3$	72	**6g**	30	600
10	**1a** (5)	5	$HS(CH_2)_3$[b]	25	**6h**	28	560
11	**1b** (5)	5	Bn	19	**6e**	84	1680
12	**1b** (5)	5	Et	22	**6f**	80	1600
13	**1b** (5)	5	$HO(CH_2)_3$	72	**6g**	49	980
14	**1b** (5)	5	$HS(CH_2)_3$[b]	25	**6h**	60	1200

[a] Although optically active L-serine-*O*-carbonate was employed as a starting material, the products **6a**–**h** were obtained as a racemic form. [b] Dithiol (0.6 eq.) was employed and the both thiol groups were alkylated.

Fig. 4 Catalytic β-replacement reaction of **7** with thiols.

Table 2 Catalytic β-replacement reaction of Ser amide and peptides

Run	Compd. **7**	X	RSH	Product[a]	Yield (%)
1	**a**	Bn	Bn	**8a**	81
2	**b**	L-Ala-OBn	Bn	**8b**	76
3	**c**	L-Val-OBn	Bn	**8c**	70
4	**d**	L-Ala-L-Ala-OBn	Bn	**8d**	68
5	**b**	L-Ala-OBn	Ph	**9**	81
6	**b**	L-Ala-OBn	Et	**10**	70

[a] Products **8b**–**d** were obtained as a mixture of diastereomers based on the cysteine residue.

Encouraged by the result described above, we applied the reaction to peptides having a serine-*O*-carbonate residue at the N-terminal position (Fig. 4),[6] and the results are summarized in Table 2. Although longer reaction time (*ca.* 100 h) was required, serine amide **7a** was converted into cysteine amide **8a** (run 1). Dipeptides **7b**, **c** and tripeptide **7d** were also transformed into the corresponding peptides bearing *S*-benzylcysteine at the N-terminal position (runs 2–4). Phenyl and ethyl mercaptans also reacted with **7b** to give **9** and **10**, respectively (runs 5 and 6).

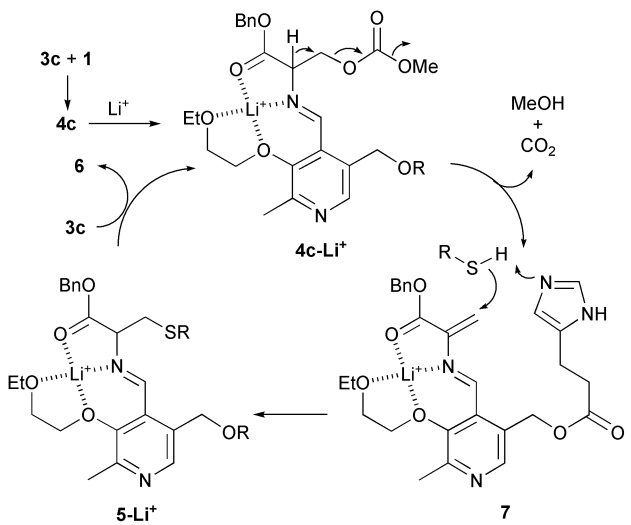


Fig. 5 Proposed reaction mechanism.

A possible reaction mechanism of the present catalytic reaction is proposed in Fig. 5. The first step would involve formation of the aldimine **4c** from **1** and **3c**. As the reaction did not proceed without Li^+, chelation of Li^+ plays a crucial role in the reaction and appears to restrict the conformation of the imino-ester moiety as shown by **4c**–**Li**$^+$,[5] which would consequently activate the elimination of the carbonate group to form unsaturated species **7**. Michael addition of a thiol, which would be activated by the imidazole moiety in the case of **1b**,§ would take place to afford cysteine aldimine **5**–**Li**$^+$. Eventually, transimination of **5**–**Li**$^+$ with **3c** is thought to give **4c**–**Li**$^+$ and the cysteine derivative **6**, forming a catalytic cycle.

In conclusion, an efficient and catalytic conversion of the serine-*O*-carbonate ester to various *S*-substituted cysteine derivatives was achieved in one pot by employing pyridoxal derivatives **1a** and **b**, which is the first example mimicking CBS. Further applications of this reaction system to other nucleophiles than thiols and to a chiral system are also in progress in our laboratory.

This work was financially supported by a SUNBOR Grant.

Notes and references

† We studied the effect of the solvent, and found that, although the reaction proceeded in other solvents, MeCN was the most effective.

‡ Pyridoxal model compound **1b** was synthesized by esterification of a pyridoxal derivative with *N*-tosylimidazole-4-propanoic acid, details of which will be reported in a full article.

§ We disclosed that the imidazole moiety does not activate the elimination of the carbonate, but activates the addition procedure, details of which will be described in a full article.

1 For review article: K. Murakami, J. Kikuchi, Y. Hisaeda and O. Hayashida, *Chem. Rev*, 1996, **96**, 728.

2 W. Weiner, J. Winkler, S. C. Zimmerman, A. W. Czarnik and R. Breslow, *J. Am. Chem. Soc.*, 1985, **107**, 4093; Y. Murakami, J. Kikuchi and T. Kitazaki, *J. Chem. Soc., Chem. Commun.*, 1998, 143; Y. Murakami, J. Kikuchi, Y. Hisaeda, K. Nakamura, T. Kitazaki and H. Kaya, *Bull. Chem. Soc. Jpn.*, 1990, **63**, 2339; Y. Murakami, Y. Hisaeda, K. Nakamura and J. Kikuchi, *Chem. Lett.*, 1990, 1765; Y. Murakami, Y. Hisaeda, T. Miyajima, H. Sakata and J. Kikuchi, *Chem. Lett.*, 1993, 645; Y. Murakami, Y. Hisaeda, T. Miyajima and J. Kikuchi, *Chem. Lett.*, 1993, 2093.

3 For example: L. Davis and D. E. Metzler, in *The Enzymes*, ed. P. D. Boyer, Academic Press, New York and London, 1972, vol. VII, pp. 1–22.

4 H. Refsum, P. M. Ueland, O. Nygard and S. E. Vollset, *Annu. Rev. Med.*, 1998, **49**, 31.

5 K. Miyashita, H. Miyabe, C. Kurozumi and T. Imanishi, *Chem. Lett.*, 1995, 487; K. Miyashita, H. Miyabe, C. Kurozumi, K. Tai and T. Imanishi, *Tetrahedron*, 1996, **52**, 12125.

6 Examples for direct modification of peptides at the N-terminal position: M. J. O'Donnell, C. Zhou and W. L. Scott, *J. Am. Chem. Soc.*, 1996, **118**, 6070; W. L. Scott, C. Zhou, Z. Fang and M. J. O'Donnell, *Tetrahedron Lett.*, 1997, **38**, 3695; K. Miyashita, H. Iwaki, K. Tai, H. Murafuji and T. Imanishi, *Chem. Commun.*, 1998, 1987; K. Miyashita, H. Iwaki, K. Tai, H. Murafuji, N. Sasaki and T. Imanishi, *Tetrahedron*, 2001, **57**, 5773.