Pergamon

Tetrahedron Letters 41 (2000) 5379–5381

TETRAHEDRON LETTERS

Studies of the enzymatic synthesis of *N*-protected amino acid-estradiol derivatives in an organic solvent

Ai-Xin Yan,[a] Guo-Wen Xing,[a,†] Yun-Hua Ye,[a,*] Gui-Ling Tian,[a] Man-Sau Wong[b] and Kin-Sing Lee[b]

[a]*Key Laboratory of Bioorganic Chemistry and Molecular Engineering, Department of Chemistry, Peking University, Beijing 100871, China*

[b]*Open Laboratory of Chirotechnology and Department of Applied Biology and Technology, The Hong Kong Polytechnic University, Kowloon, Hong Kong, China*

Received 14 March 2000; accepted 16 May 2000

Abstract

Amino acid-estradiol derivatives were synthesized via protease-catalyzed condensation for the first time and the optimum conditions were studied systematically.

Peptidyl steroids include those compounds in which amino acids or peptides are coupled with steroids to form amides or esters. Cholyl glycine and cholyl taurine are examples of peptidyl steroids that play important roles in the process of human life.[1] In the previous paper, a series of amino acid and peptide derivatives of estradiol has been synthesized using different coupling reagents and their binding affinities for the estrogen receptor have been studied.[2] In this paper, we present the results of a study of the synthesis of this kind of compound catalyzed by a protease in an organic solvent. The advantages of enzymatic methods for peptidyl steroid synthesis include the use of mild reaction conditions, which are generally racemization free, minimal side chain protection and high regio- and stereoselectivity.[3] Acyl donors with different structures, such as different *N*-protecting groups and different esters were studied and are discussed.

P-L-Ala-OR was chosen as the acyl donor and the 17-aminoestra-1,3,5(10)-trien-3-ol (I) or 17-hydrazonoestra-1,3,5(10)-trien-3-ol (II) as the acyl acceptor substrate. The amide bond formation between P-L-Ala-OR and the 17-β-amino or 17-β-hydrazono group of estradiol (I,II) was catalyzed by subtilisin Carlsberg in DMF containing a little water (Fig. 1).

* Corresponding author. E-mail: yhye@pku.edu.cn

† Present address: Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences.

0040-4039/00/$ - see front matter
PII: S0040-4039(00)00797-8

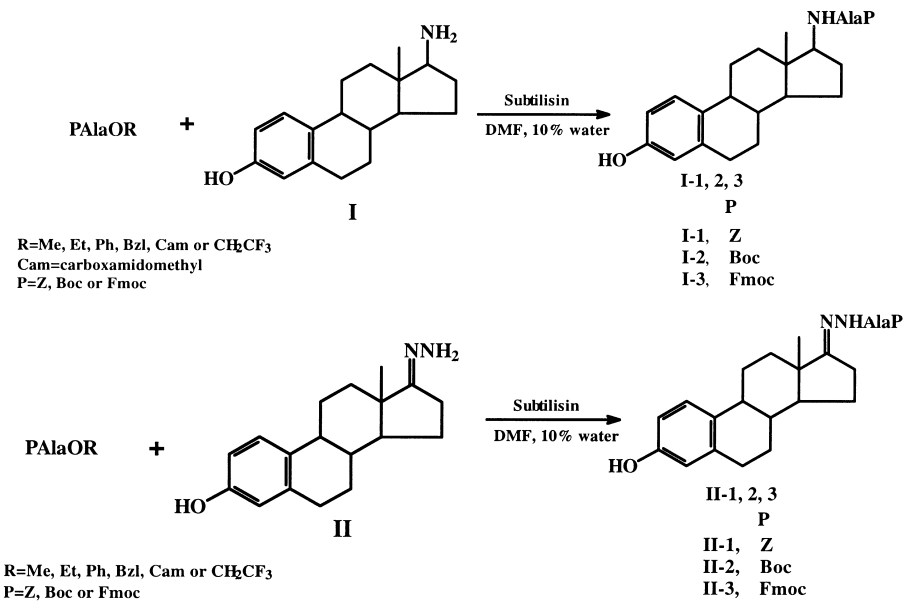

Figure 1. Synthetic scheme for P-Ala-estradiol derivatives

In order to investigate the effects of different carboxyl components on the yield of the enzymatic reactions, a series of *N*-protected alanine esters were prepared[4–6] and condensed with compound **I** or **II**. The results are displayed in Tables 1 and 2.

Table 1
Effects of different protecting groups on the yield of the enzymatic reactions

acyl donor	Nucleophile	Product	Yield%	m.p. (•)	$[\alpha]_D^{19}$ (c, sol.)	FAB-MS
$ZAlaOCH_2CF_3$	I	I-1	35	108-110	–16.7(0.5, EtOH)	$477(M+H)^+$
$BocAlaOCH_2CF_3$	I	I-2	77	129-131	–31.0(0.5, EtOH)	$443(M+H)^+$
$FmocAlaOCH_2CF_3$	I	I-3	0	--	--	--
$ZAlaOCH_2CF_3$	II	II-1	56	173-176	+56.8(0.985, DMF)	$490(M+H)^+$
$BocAlaOCH_2CF_3$	II	II-2	87	174-176	+120.5(0.41, DMF)	$456(M+H)^+$
$FmocAlaOCH_2CF_3$	II	II-3	40	147-150	+95.2(0.32, EtOH)	$578(M+H)^+$

Table 2
Effects of different esters on the enzymatic reaction

acyl donor	Product	Yield %
$Boc\text{-}Ala\text{-}OCH_3$	I-2	0
$Boc\text{-}Ala\text{-}OCH_2CH_3$	I-2	0
Boc-Ala-OPh	I-2	24
Boc-Ala-OBzl	I-2	trace
Boc-Ala-OCam	I-2	trace
$Boc\text{-}Ala\text{-}OCH_2CF_3$	I-2	77

As shown in Table 1, Boc was better as the amino protecting group for the acyl donor than *Z* or Fmoc.‡ Since Boc has the smallest size among the three blocking groups, this result suggests that subtilisin Carlsberg has a small hydrophobic pocket in its S_2 position and Boc is more suitable for the binding between substrate and subtilisin. Table 1 also demonstrates that compound II acted as a better nucleophile than compound I due to favorable electronic effects. Table 2 indicates that the trifluoroethyl ester is the best substrate to be used as the carboxyl component for the enzymatic reaction. Furthermore, it seemed that electron-withdrawing groups such as trifluoroethyl, phenyl, benzyl and carboxamidomethyl in the carboxyl component were preferable to electron-donating groups such as methyl and ethyl esters. This result suggests that electronic effects play an important role in the acyl-enzyme intermediate formation process. A previous report[7] also recommended the use of trifluoroethyl esters of amino acids as the carboxyl components due to their ability to facilitate the process of acyl-enzyme formation, the key step in enzymatic condensation.

The optimum molar ratio for the enzymatic synthesis was also studied with the coupling between Boc-Ala-OCH_2CF_3 and compound **I** as a model reaction. The best result was gained when two equivalents of the carboxyl component were used.

In conclusion, we have successfully obtained *N*-protected amino acid-estradiol derivatives catalyzed by subtilisin Carlsberg in DMF. The structures of all the products were confirmed by FAB-MS, ^{13}C NMR, $[\alpha]_D$ and elemental analysis. These results indicate that the estradiol derivatives (I and II) are compatible as nucleophilic substrates for subtilisin and can provide useful information for broadening the use of protease-catalyzed reactions for routine organic synthesis.

Acknowledgements

This work was supported by the Hong Kong Polytechnic University and National Natural Science Foundation of China.

References

1. Pettit, G. R.; Das Gupta, A. K.; Smith, R. L. *Can. J. Chem.* **1966**, *44*, 2023.
2. Ye, Y. H.; Huang, Y. S.; Wang, Z. Q.; Chen, S. M.; Tian, Y. *Steroids* **1993**, *58*, 35.
3. Koskinen, A. M. P.; Klibanov, A. M. *Enzymatic Reactions in Organic Media*; Blackie Academic & Professional: London, 1996.
4. Chen, Sh. T.; Wang, K. T. *J. Chem. Soc., Chem. Commum.* **1988**, 327.
5. Morira, A. B.; Singh, B.; Ester, F. *J. Am. Chem. Soc.* **1977**, *99*, 8491.
6. Martinez, J.; Laur, J.; Castro, B. *Tetrahedron Lett.* **1983**, *24*, 5219–5222.
7. Miyazawa, T.; Tanaka, K.; Ensatsu, E.; Yanagihara, R.; Yamada, T. *Tetrahedron Lett.* **1998**, *39*, 997.

‡ Abbreviations. Boc: *tert*-Butyloxycarbonyl; *Z*: Benzyloxycarbonyl; Fmoc: 9-Fluorenylmethyloxycarbonyl.