

SYNTHESIS OF TRIS-BRIDGED CYCLIC PEPTIDES AS AN ACTIVE SITE MIMIC OF LIPASE

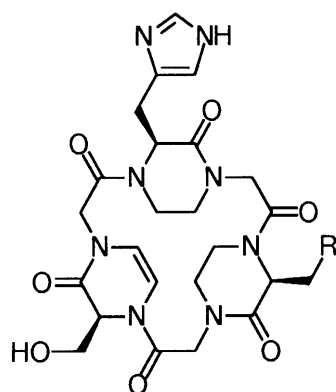
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Tris-bridged cyclic peptides containing serine-histidine and carboxylic acid as an active site of lipase were synthesized. The catalytic activity of the synthetic peptides for the transesterification of 4-nitrophenyl acetate in DMSO was examined.

KEY WORDS lipase; active site mimic; cyclic peptide; hydrophobic surroundings; triad

In an earlier communication, we reported the synthesis of a tris-bridged cyclic hexapeptide (**1a**) which bears the serine-histidine as the catalytic site residue of lipase¹). Lipase has been widely used for asymmetric hydrolysis and esterification²). Recently, the structure of lipase from various sources has been revealed³). Common to the family of hydrolase enzymes is the catalytic triad of a nucleophile-histidine-acidic residue, which is observed in the lipases as either a serine-histidine-aspartic acid triad or a serine-histidine-glutamic acid triad. In this paper, we describe the synthesis of the tris-bridged cyclic peptides (**1b-f**) which bear the serine-histidine and carboxylic acid as the catalytic site residues and contain the three bridge units which connect all nitrogens of backbone amides to fix the direction of the catalytic triad residues. The tris-bridged cyclic peptides containing carboxylic acid are found to accelerate the transesterification of 4-nitrophenyl acetate.



1 a : R=CONH₂

1 b : R=CO₂H

1 c : R=CH₂CO₂H

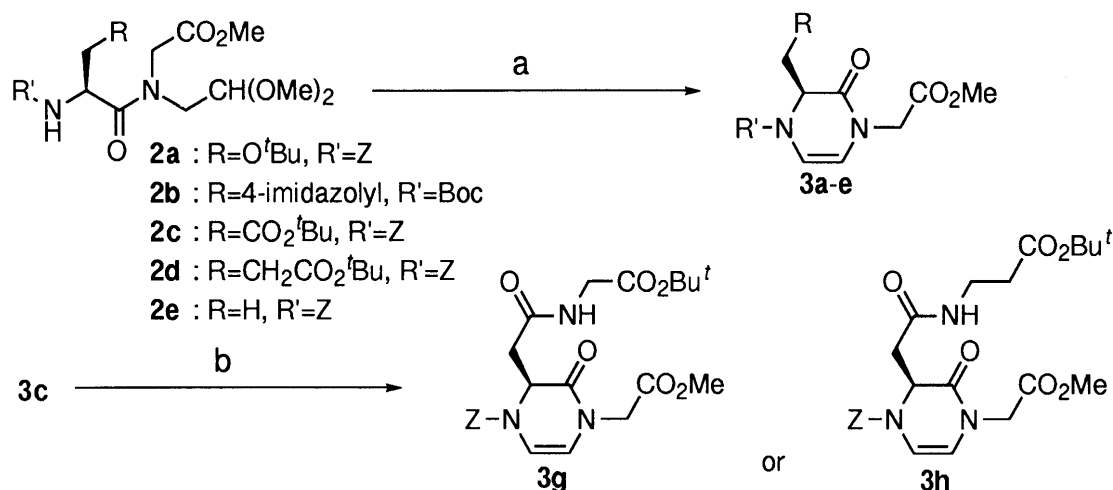
1 d : R=CONHCH₂CO₂H

1 e : R=CONH(CH₂)₂CO₂H

1 f : R=H

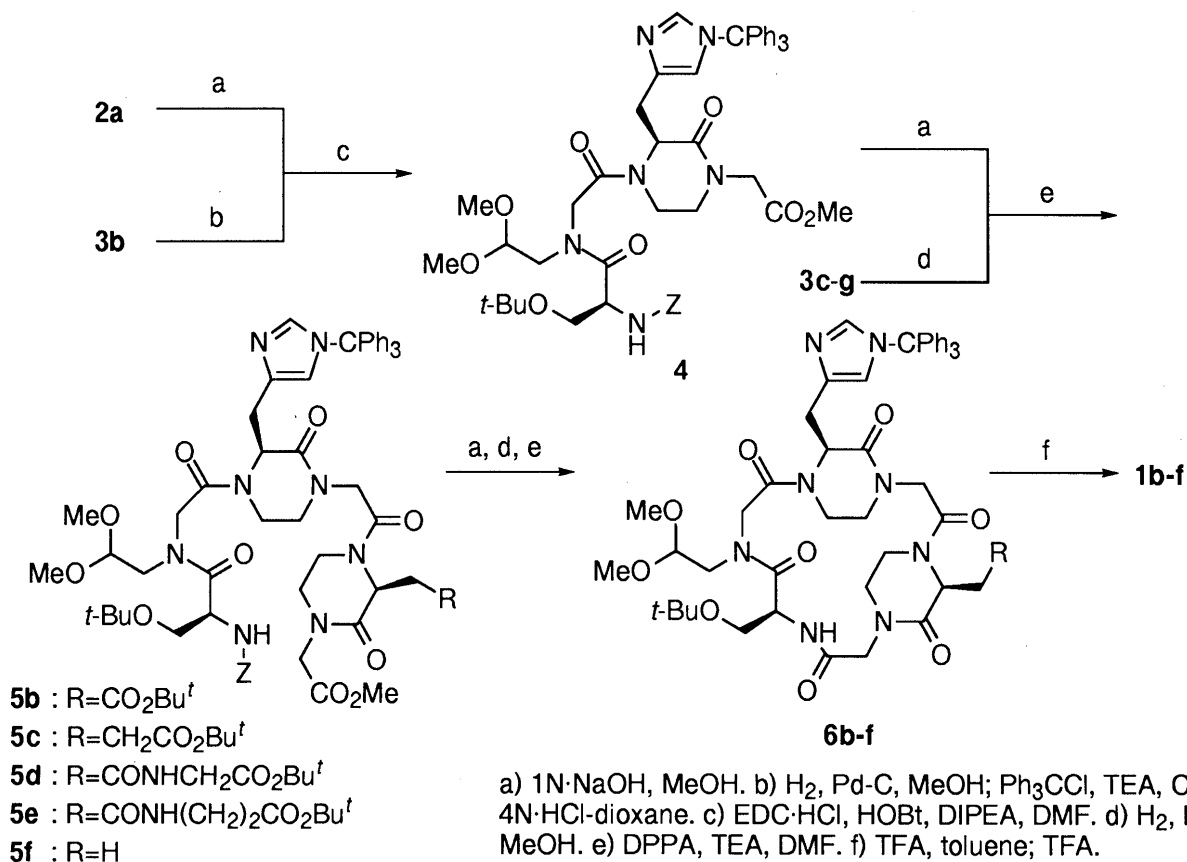
The tris-bridged cyclic peptides consist of three units of the piperazine-2-one derivatives which were synthesized from the substituted dipeptides *via* acid-catalyzed cyclization. The carboxylic acid, which was obtained by the deprotection of **3c** with trifluoroacetic acid (TFA), was coupled with glycine *tert*-butyl ester or β -alanine *tert*-butyl ester using ethyl((dimethylamino)propyl)carbodiimide-HCl (EDC·HCl) and hydroxybenzotriazole (HOBt) to give the piperazine-2-one derivatives (**3f**, **3g**). After the deprotection of tetrapeptide (**4**), which was prepared from seryl glycine derivative **2a** and

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a) *p*-TsOH or TFA, toluene. b) TFA; glycine *tert*-butyl ester or β -alanine *tert*-butyl ester, EDC·HCl, HOBT, DMF.

histidyl glycine derivative **3b**, with 1N·NaOH-methanol, the resultant tetrapeptide carboxylic acid was coupled with the N-deprotected piperazin-2-one derivative (**3c-g**), respectively. Two-step deprotection, followed by cyclization with DPPA gave the bis-bridged cyclic peptides (**6b-f**). Finally, the bis-bridged cyclic peptides (**6b-f**) were treated with TFA to obtain the tris-bridged cyclic peptides (**1b-f**)⁴.



The catalytic activity of the tris-bridged cyclic peptides (**1a-f**) for the transesterification of 4-nitrophenyl acetate was examined. Mainly because the active site of lipase is covered by a surface loop and is therefore inaccessible to solvent water, we carried out lipase-mimic-catalyzed hydrolysis

in DMSO as the hydrophobic surroundings. The second-order reaction rate constants (k_2) were calculated using equation below:

$$k_2 = k_{\text{obs}} - k_{\text{spont}}$$

where the observed reaction rate constant (k_{obs}) and the spontaneous rate constant (k_{spont}) were obtained by HPLC observing the generated 4-nitrophenol. Our results are summarized in Table 1.

Table 1. Second-order Rate Constants ($10^8 k_2$, $\text{mol}^{-1} \text{dm}^3 \text{s}^{-1}$) for the Transesterification of 4-Nitrophenyl Acetate by Tris-bridged Cyclic Peptides at 35°C

Entry	1	H ₂ O (M) in DMSO			
		0	0.5	1	5
1	1a	1.2	-	-	-
2	1b	9.8	-	-	-
3	1c	18.7	14.5	12.3	5.7
4	1d	13.2	11.2	10.4	6.8
5	1e	13.1	-	-	-
6	1f	3.5	3.6	3.5	2.8

[4-nitrophenyl acetate]= $1.0 \times 10^{-3} \text{mol dm}^{-3}$; [1]= $1.0 \times 10^{-3} \text{mol dm}^{-3}$; in DMSO or H₂O-DMSO.

Compound **1f**, in which the carboxylic acid part of **1b-e** was replaced by methyl piperizin-2-one (bridged alanyl glycine), exhibited less activity than **1b-e**. These results indicate that carboxylic acid plays an important role in the acceleration of the transesterification reaction, although the activity of **1f** is not observed in DMSO. It seems that the increase of water molecule in DMSO decreases the stability of the hydrogen bond relay system of the nucleophile-histidine-acidic residue catalytic triad.

In conclusion, we have achieved the synthesis of the tris-bridged cyclic peptides (**1b-e**) containing serine-histidine and carboxylic acid as an active site of lipase. Compounds **1b-e**, which bear the carboxylic acid, were found to accelerate the transesterification of 4-nitrophenyl acetate. Reactions by lipase active-site mimics in the hydrophobic surroundings of DMSO show the effectiveness of the relay system of serine-histidine-carboxylic acid. These results indicate that the enzymatic reaction solvent is very important in examining the catalytic activity of enzyme mimics.

REFERENCES AND NOTES

- 1) Uchida H., Achiwa K., *Synlett*, **1996**, 969-970.
- 2) A recent review, see: Santaniello E., Ferraboschi P., Grisenti P., Manzocchi A., *Chem. Rev.*, **92**, 1071-1140 (1992).
- 3) Kohno M., Funatsu J., Mikami B., Kugimiya W., Matsuo T., Morita Y., *J. Biochem.* **120**, 505-510 (1996), and references cited therein.
- 4) **1b**: amorphous powder, $[\alpha]_{\text{D}}^{24} + 49.8^\circ$ (c=0.2, MeOH), MS: m/z 587(M+1)⁺, 609(M+Na)⁺.
1c: amorphous powder, $[\alpha]_{\text{D}}^{27} + 25.8^\circ$ (c=0.3, MeOH), MS: m/z 601(M+1)⁺, 623(M+Na)⁺.
1d: amorphous powder, $[\alpha]_{\text{D}}^{30} + 37.7^\circ$ (c=0.3, MeOH), MS: m/z 644(M+1)⁺, 666(M+Na)⁺.
1e: amorphous powder, $[\alpha]_{\text{D}}^{27} + 35.8^\circ$ (c=0.4, MeOH), MS: m/z 658(M+1)⁺, 680(M+Na)⁺.
1f: amorphous powder, $[\alpha]_{\text{D}}^{23} + 37.7^\circ$ (c=0.4, MeOH), MS: m/z 543(M+1)⁺.

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