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Lipase-catalyzed Regioselective Synthesis of Vinyl Ester Derivatives of Thiamphenicol: Novel Thiamphenicol Monomers for Preparation of Macromolecular Antibiotic

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Abstract: Three polymerizable vinyl thiamphenicol esters with different acyl donor carbon chain length (C4, C6, C10) were regioselectivly synthesized by Lipozyme® (immobilized from *mucor miehei*) in acetone at 50 ℃ for 12 h to give 73%, 81%, 63% yield, respectively. The products were valuable monomers for preparation of macromolecular antibiotic.

Keywords: Thiamphenicol, vinyl ester, transesterification, regioselectivity, monomer.

Macromolecular drugs have attracted considerable interests as they can effectively control the rate of the drug release and increase the therapeutic benefit[1]. Synthesis of drug monomers is an important step for macromolecular drugs preparation. Recently various structural modification of drugs using chemical[2] or enzymatic[3] method has been reported. While few products of modified drugs possesses polymerizable groups, which can be used for the preparation of macromolecular drugs. We have focused on exploiting enzymatic or chemo-enzymatic methods for the preparation of macromolecular drugs such as enzymatic synthesis of vitamin C, acyclovir[4], chloramphenicol[5] and guaifenesin[6] polymeric drugs or monomers. Thiamphenicol (D(+)-*threo*-1-(4'-methyl sulphonyl-phenyl)-2-dichloroacetamido-propane-1, 3-diol) is characterized by a broad-spectrum antibiotic activity similar to chloramphenicol, with lower toxicity and higher activity than chloramphenicol[7]. As a part of macromolecular thiamphenicol preparation, in the present study we synthesized three polymerizable vinyl thiamphenicol esters monomers bearing acyl donor with different carbon chain length (C4, C6, C10). The reaction was catalyzed by lipozyme® under mild conditions and in good regioselectivity and yields.

Experimental

Lipozyme® was purchased from Fluka (62 U/g, Switzerland). Acetone was dried over 3 Å molecular sieves for 24 h *prior to* use. Divinylsuccinate, divinyladipate, divinyl sebacate were produced and purified as described by the literature[8]. Thiamphenicol, vinyl acetate and all other chemicals were of the purity commercially available.

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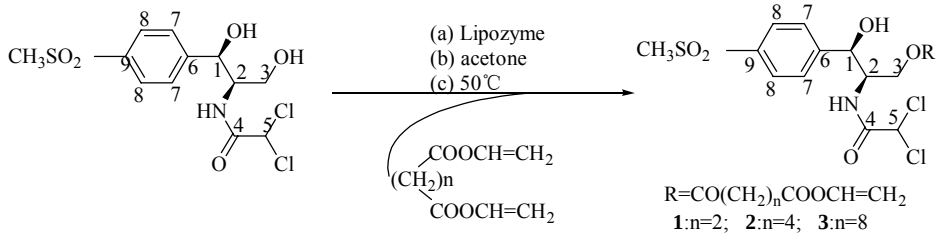
Scheme 1 Enzymatic synthesis of vinyl thiamphenicol esters (**1**, **2**, **3**)



Thiamphenicol Vinyl thiamphenicol esters

The process of the reaction was monitored by TLC with an eluent of ethyl acetate/petroleum ether (2/1, v/v). Infrared spectra were measured with a Nicolet Nexus FTIR 670. The position of acylation in enzymatically prepared thiamphenicol esters was established by ^{13}C-NMR (Bruker AVANCE DMX 500).

Thiamphenicol 0.5 g (0.001 mol) was dissolved in 20 mL acetone, divinyl dicarboxylates (C4, C6, C10) (0.004 mol) were added. The reaction was initiated by adding 0.3 g Lipozyme® and the suspension was shaken at 50 ºC for 12 h. The reactions were terminated by filtering off the enzyme. The acetone was evaporated. Formation of the vinyl thiamphenicol ester was confirmed by TLC. Products were isolated by silica gel column chromatography with the eluent consisting of ethyl acetate/petroleum ether (2/1, v/v). Enzymatic synthesis of vinyl thiamphenicol esters were shown in **Scheme 1**.

Results and Discussion

To choose the more efficient enzyme and suitable solvent on the synthesis of vinyl thiamphenicol esters, five commercially available enzymes (Alkaline protease from *Bacillus subtilis*, Lipozyme®, Lipase from Porcine pancreas, Lipase from *Candida cylindracea*, and Lipase from Hog pancreas) and four solvents (acetone, pyridine, acetonitrile, DMF) were tested for the transesterification of thiamphenicol with divinyladipate. The best result was obtained from Lipozyme® in acetone and the yield was 81%, while the lipase from *Candida cylindracea* showed the lowest activity to catalyze the reaction in acetone.

The obtained thiamphenicol vinyl esters were characterized by FTIR and NMR spectroscopy[9]. The position of enzymatic acylation in thiamphenicol vinyl esters was established by ^{13}C-NMR, as shown in **Table 1 (1, 2, 3)**. The general strategy was the same as described by Yoshimoto *et al.*[10]. As established by them, acylation of a hydroxyl group of substrate resulted in a downfield shift of the peak of the *O*-acylated carbon and an upfield shift of the peak of the neighboring carbon. Characterization of the products (**1, 2, 3**) by ^{13}C-NMR revealed that vinyl thiamphenicol esters were substituted at C-3 position of thiamphenicol. Thus signals for C-3 of thiamphenicol shifted downfield from 62.3 ppm to 63.1 ppm and C-2 position shifted upfield from 58.2 ppm to 54.4 ppm. Furthermore, the ^{1}H-NMR of the corresponding thiamphenicol derivatives also confirmed the regioselective acylation at the primary OH (downfield shift of the signal). The results

imply that Lipozyme® shows an effective regioselectivity in the esterification of thiamphenicol with divinyl dicarboxylates (C4, C6, C10).

Table 1 Chemical shifts of ^{13}C-NMR ($CDCl_3$ δ ppm) of thiamphenicol and its vinyl esters

Carbon number	Thiamphenicol	**1**	**2**	**3**
1	71.44	70.78	70.89	70.95
2	58.18	54.47	54.41	54.45
3	62.31	63.13	63.12	62.83
4	164.65	164.58	164.56	164.51
5	67.70	66.34	66.35	66.35
6	141.15	141.14	141.26	141.39
7	128.04	127.76	127.59	127.68
8	127.97	127.14	127.17	127.14
9	149.75	146.78	147.12	146.86
10	44.53	44.79	44.74	44.77
─CH_2		29.04	33.92	34.30
		28.99	33.64	34.12
			24.37	29.20
			24.08	29.12
				24.99
				24.74
C═O		172.67	173.57	174.35
		170.16	170.86	171.20
		164.58	164.56	164.51
─CH=		140.13	139.82	140.02
		98.75	98.21	97.85

The obtained vinyl thiamphenicol esters would act as useful monomers for the synthesis of high potency, hypotoxicity, controlled release polymer drugs. We have reported enzymatic synthesis of vinyl sugar esters and preparation of polymers containing sugar branches[11, 12]. Copolymerization of vinyl thiamphenicol esters with vinyl sugar esters can prepare novel macromolecular thiamphenicol antibiotic with hydrophilic property and targeting group. Preparation of macromolecular antibiotic containing thiamphenicol and sugar branches is in progress.

References and Notes

1. R. A. Gross, A. Klumar, B. Kalra, *Chem. Rev.*, **2001**, *101*, 2097.
2. E. H. Abdennebi, R. J. Sawchuk, C. M. Stowe, *J. Vet. Pharmacol. Ther.*, **1994**, *17*, 365.
3. G. Ottolina, G. Carrea, S. Riva, *J. Org. Chem.*, **1990**, *55*, 2366.
4. X. T. Xue, D. S. Lu, Z. C. Chen, Q. Wu, Y. Cai, X. F. Lin, *Chin. Chem. Lett.*, **2003**, *14*(2), 163.
5. D. S. Lu, X. T. Xue, N. Wang, Q. Wu, X. F. Lin, *Prep. Biochem. Biotech.*, **2004**, *34*(1), 95.
6. N. Wang, Q. Wu, B. K. Liu, Y. Cai, X. F. Lin, *J. Mol. Catal. B-Enzym.*, **2004**, *27*(2), 97.
7. D. Becker, J. Katz, J. Metz, *Postgrad. Med. J.*, **1974**, *50*(Suppl 5), 88.
8. E. D. M. John, W. S. Henry, J. P. Robert, *British Patent*, **1960**, 827718.

9. 3-*O*-Vinylsuccinyl-thiamphenicol (**1**): White solid, M.p. 113-115 ºC. Yield: 73%. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.90 (d, 2H, J=8.25 Hz) and 7.59 (d, 2H, J=8.19 Hz), aromatic protons; 6.95 (d, 1H, -NHCO); 5.79 (s, 1H, -$CHCl_2$); 5.12 (d, 1H, J=2.15 Hz, Ph-CH-); 4.52 (dd, 1H, J=5.6 and 10.99 Hz, CH_2-O); 4.36 (dd, 1H, J=8.2 and 10.99 Hz, CH_2-O); 4.25 (ddd, 1H, J=2.63, 5.43 and 8.23 Hz, CH-NHCO); 3.04 (s, 3H, CH_3SO_2); 2.79 (t, 2H), 2.71 (t, 2H), succinyl part; 7.25 (dd, 1H, J=6.32 and 13.99 Hz, -CH=), 4.94 (d, 1H, CH_2=, J=12.19 Hz), 4.63 (d, 1H, CH_2=, J= 4.03 Hz), vinyl moiety. IR (KBr, cm^{-1}): 1762 (C=O); 1643 (C=C); 1597, 1537, (arom); 1147 (SO_2); 1209 (C-O).
   3-*O*-Vinyladipoyl-thiamphenicol (**2**): White solid, M.p. 115-116 ºC. Yield: 81%. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.78 (d, 2H, J=7.93 Hz) and 7.53 (d, 2H, J=8.04 Hz), aromatic protons; 6.99 (d, 1H, -NHCO); 5.79 (s, 1H, -$CHCl_2$); 5.07 (d, 1H, J=2.4 Hz, Ph-CH-); 4.41 (dd, 1H, J=5.4 and 10.8 Hz, CH_2-O); 4.35 (dd, 1H, J=8.1 and 10.1 Hz, CH_2-O); 4.22 (ddd, 1H, J=2.9, 5.6 and 8.5 Hz, CH-NHCO); 3.02 (s, 3H, CH_3SO_2) 2.42 (m, 4H), 1.70 (m, 4H), adipoyl part; 7.24 (dd, 1H, J=6.15 and 13.99 Hz, -CH=), 4.89 (d, 1H, J=13.79 Hz, CH_2=), 4.59 (d, 1H, J=6.37 Hz, CH_2=), vinyl moiety. IR (KBr, cm^{-1}): 1740 (C=O); 1647 (C=C); 1596, 1545 (arom); 1144 (SO_2); 1198 (C-O).
   3-*O*-Vinylsebacoyl-thiamphenicol (**3**): White solid, M.p. 74-76 ºC. Yield: 63%. ^{1}H-NMR ($CDCl_3$, δ ppm): 7.82 (d, 2H, J=7.38 Hz) and 7.54 (d, 2H, J=8.06 Hz), aromatic protons; 6.94 (d, 1H, -NHCO); 5.78 (s, 1H, -$CHCl_2$); 5.05 (d, 1H, J=2.27 Hz, Ph-CH-); 4.44 (dd, 1H, J=5.77 and 11.09 Hz, CH_2-O); 4.36 (dd, 1H, J=8.07 and 11.00 Hz, CH_2-O); 4.20 (ddd, 1H, J=2.23, 5.40 and 8.52 Hz, -CH-NHCO); 3.04 (s, 3H, CH_3SO_2) 2.37 (m, 4H), 1.63 (m, 4H), 1.32 (m, 8H), sebacoyl part; 7.26 (dd, 1H, J=7.44 and 14.02 Hz, -CH=), 4.88 (d, 1H, J=12.80 Hz, CH_2=), 4.57 (d, 1H, J=5.07 Hz, CH_2=), vinyl moiety. IR (KBr, cm^{-1}): 1757 (C=O); 1645 (C=C); 1599, 1532 (arom); 1152 (SO_2); 1211 (C-O).
10. K. Yoshimoto, Y. Itatani, Y. Tsuda, *Chem. Pharm. Bull*., **1980**, *28*, 2065.
11. Q. Wu, J. Y. Feng, C. G. Feng, D. S. Lu, X. F. Lin, *Chin. Chem. Lett*.**, 2002**, *13*(5), 416.
12. Q. Wu, D. S. Lu, Z. C. Chen, G. H. Yu, X. F. Lin, *Chem. J. Chin. Univ.,* **2003**, *24*(10), 1806.

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