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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6687–6690
Bioorganic & Medicinal Chemistry Letters

Highly regioselective enzymatic synthesis of polymerizable derivatives of methyl shikimate

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Received 29 July 2007; revised 15 October 2007; accepted 17 October 2007
Available online 22 October 2007

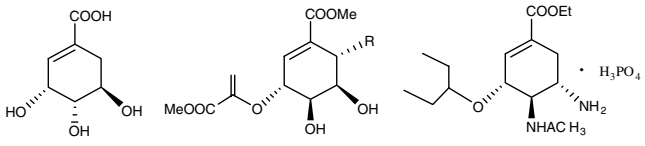
Abstract—Regiocontrollable selectivity of enzymatic method for synthesis of polymerizable derivatives of methyl shikimate was described. Lipase acrylic resin from *Candida antarctica* (CAL-B) and immobilized lipase from *Mucor miehei* (MML) showed high regioselectivity toward the secondary hydroxyl of methyl shikimate, which presents three hydroxyl groups with similar reactivity. Catalysis by MML in acetone facilitated the single step synthesis of 5-*O*-acyl methyl shikimate derivatives in high yields, while the use of CAL-B in acetone afforded 4-*O*-acyl methyl shikimate derivatives. The obtained series of methyl shikimate derivatives would be important monomers for potential useful analogues of shikimic acid.

As a key intermediate of many hydroaromatic and aromatic compounds, shikimic acid (**1**) plays an important role in the metabolism of carbohydrates as well as biosynthesis of folate coenzymes and essential aromatic amino acids L-phenylalanine, L-tyrosine, and L-tryptophan.[1] It is an analogue of numerous natural products such as quinic acid, cyathiformines B–D (**2**–**4**), and various isoprenoid quinones, which present interesting biological and pharmaceutical properties.[2] Additionally, shikimic acid is an important starting material for the synthesis of neuraminidase inhibitors such as the antiinfluenza agent Tamiflu (**5**) (Scheme 1).[3] Therefore, analogues of shikimic acid have attracted much attention and are considered to be potential antibacterial, antiviral, and antiparasitic agents.[4,5]

Regioselective modification of one hydroxyl group of shikimic acid is an interesting but difficult challenge due to several hydroxyl groups with similar reactivity presenting in the structure of shikimic acid, and a clear discrimination between them still remains a difficult task.[6,7] The substrate often reacts with more than one hydroxyl group, thus inevitably leading to a mixture of mono-, di- or multi-substituted products. Generally, conventional chemical methods involving tedious, multi-step blocking/debloking strategy show an almost complete lack of regioselectivity.

Application of biocatalyst in modification of polyfunctional compounds has been proved to be one of the most attractive alternatives to the conventional chemical methods due to its high selectivity and simplicity.[8–10] Enzyme-catalyzed reactions offer a highly efficient process under mild conditions, diminish undesired side-reactions, and facilitate product recovery.[11] Furthermore, enzymes are proteins, and as such they are completely biodegradable and environmentally acceptable. As a result, this method has been extensively used for the modification of carbohydrates, nucleosides, and other natural products.[12–14]

The use of lipase in non-aqueous solvents has been previously described for preparation of shikimic acid deriv-



1 **2** R=OH, **3** R=Cl, **4** R=H, **5**

Scheme 1. Structures of shikimic acid and its analogues.

Keywords: Enzymatic synthesis; Lipase; Regioselectivity; Methyl shikimate; Polymerizable.
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doi:10.1016/j.bmcl.2007.10.064

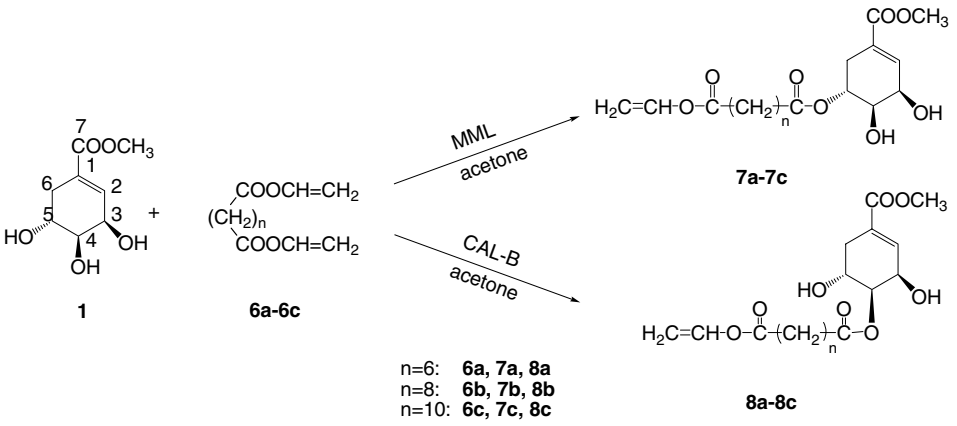
atives. Riva et al. were the first to research lipase-catalyzed regioselective acylation of quinic acid, shikimic acid, and their derivatives in organic solvents.[15] However, acylation of methyl shikimate showed no regioselectivity with any of the enzymes tested. Gotor and coworkers reported enzymatic regioselective acylation of methyl shikimate exclusively at the C-4 position hydroxyl group.[16] To our knowledge, highly regioselective enzymatic acylation of shikimic acid toward other hydroxyl positions has never been reported. Moreover, few modified shikimic acid derivatives possess polymerizable group, which can be used for copolymerization with other bioactive compounds such as sugar, amino acids, or reaction with other agents to form potential useful analogues of shikimic acid.

Herein, we describe a facile regiocontrollable method of the acylation toward the different secondary hydroxyls of methyl shikimate to obtain polymerizable derivatives. 4-*O*-acyl and 5-*O*-acyl vinyl derivatives of methyl shikimate had been prepared through two different methods, respectively. The methyl shikimate derivatives obtained would be useful as significant intermediates and monomers for preparation of useful natural products, macromolecular compounds, and bioactive derivatives.

The carboxyl present in the shikimic acid leads to low solubility and reactivity, therefore methyl shikimate was prepared in order to enhance the solubility and reaction rate. The key point of present work was to identify an appropriate enzyme catalyst suitable for acylation of methyl shikimate at different hydroxyl positions. Enzymes derived from different sources always exhibit different properties, such as specificity, activity, stability, and so on. In order to choose the appropriate enzymes to selectively catalyze the acylation toward the different hydroxyls of methyl shikimate, 10 commercially available enzymes,[17] including seven lipases and three proteases, were chosen to catalyze the transesterification for comparison in a predominant anhydrous acetone, which has lower toxicity, easier processing, and can dissolve substrates very well. The control experiment was carried out in the absence of enzyme catalyst, no product was detected by TLC and HPLC even after 96 h. After all 10 kinds of enzymes were tested, the result showed that the use of MML (Lipozyme®) facilitated the single step synthesis of 5-*O*-acyl methyl shikimate, while catalysis by CAL-B gave 4-*O*-acyl methyl shikimate. The other enzyme catalysts showed very low activity and selectivity in this acylation of methyl shikimate. The synthesis routes are shown in Scheme 2.

A typical enzymatic experimental procedure for the synthesis of **7a** and **8a** is illustrated in Scheme 2. The reaction was initiated by adding 10 mg/mL enzyme to 10 mL anhydrous acetone containing 0.3 g (1.06 mmol) methyl shikimate and 4.24 mmol divinyl adipate. The suspension was maintained at 30 °C and shaken at 200 rpm for 12 h. Formation of products was detected by TLC. The reaction was terminated by filtering out the enzyme, and the filtrate was concentrated under reduced pressure. The products were isolated by silica gel chromatography with an eluent consisting acetone/CH_2Cl_2 (1:5, v/v). The vinyl ester derivatives obtained were characterized by ^1H NMR, ^{13}C NMR (Bruker DMX400), and HRMS (Bruker Daltonics Bio TOF).[18] Other products (**7b**, **7c**, **8b**, and **8c**) were synthesized by the same methods for **7a** and **8a**. The yields of the methyl shikimate derivatives are summarized in Table 1.

To increase the selectivity exhibited by MML and CAL-B, several organic solvents were tested for the transesterification of methyl shikimate with divinyl adipate. The results are shown in Table 2. In general, solvents of log *P* between −0.5 and 0.79 are considered to be the most suitable for the reaction, such as *tert*-butyl-alcohol, THF, acetone, and dioxane. Other polar solvents, such as DMF, DMSO, are not suitable for these lipases. In contrast, apolar solvents, such as hexane, chloroform, are suitable for ester synthesis, but the reactions were hindered by low solubility of substrate. MML showed high activity and selectivity in acetone, THF, and dioxane toward the C-5 position hydroxyl group of methyl shikimate (entries 7, 9, and 13, Table 2), while CAL-B showed high yields and regioselectivity at the C-4 position in acetone (entry 10, Table 2), and the best selectivity was given in acetonitrile (entry 12, Table 2).



Scheme 2. Enzymatic synthesis of 4-*O*-acyl and 5-*O*-acyl vinyl esters of methyl shikimate.

Table 1. Regioselective enzymatic acylation of methyl shikimate

Entry	Enzyme	Acyl donor	*t* (h)	Yields of 5-*O*-acyl[a] (%)	Yield of 4-*O*-acyl[a] (%)
1	MML	**6a**	12	65.1	—
2	MML	**6b**	13	55.8	—
3	MML	**6c**	15	54.2	—
4	CAL-B	**6a**	12	Trace	57.4
5	CAL-B	**6b**	14	Trace	62.5
6	CAL-B	**6c**	14	Trace	54.3

[a] Isolated yields by silica gel chromatography, purity confirmed by HPLC, NMR, and HR-MS.

Table 2. Influence of organic solvent on selectivity and yield catalyzed by different enzymes

Entry	Solvent	Log *P*	Enzyme	Conv.[a] (%)	Yield of **7a**[a,b] (%)	Yield of **8a**[a,b] (%)	Selectivity[c] **7a/8a**
1	Hexane	3.9	MML	17.8	5.2	10.8	37:63
2	Hexane	3.9	CAL-B	15.2	8.6	5.4	61:39
3	Chloroform	2.0	MML	21.6	6.7	13.5	33:67
4	Chloroform	2.0	CAL-B	33.6	12.6	17.7	42:58
5	*tert*-Butyl- alcohol	0.79	MML	67.5	52.6	4.4	92:8
6	*tert*-Butyl- alcohol	0.79	CAL-B	40.1	4.4	31.7	13:87
7	THF	0.46	MML	79.1	71.4	3.2	96:4
8	THF	0.46	CAL-B	50.6	5.0	41.2	11:89
9	Acetone	−0.23	MML	82.4	76.1	5.4	93:7
10	Acetone	−0.23	CAL-B	88.7	8.0	74.9	10:90
11	Acetonitrile	−0.39	MML	35.2	28.9	6.0	83:17
12	Acetonitrile	−0.39	CAL-B	55.4	2.3	45.2	5:95
13	Dioxane	−0.5	MML	75.9	69.7	1.7	98:2
14	Dioxane	−0.5	CAL-B	38.5	2.2	33.6	6:94
15	DMF	−1.0	MML	4.5	3.2	0	100:0
16	DMF	−1.0	CAL-B	9.1	4.2	4.5	48:52
17	DMSO	−1.3	MML	2.2	1.5	0	100:0
18	DMSO	−1.3	CAL-B	3.5	0	3.1	0:100

Conditions: MML (10 mg/mL) or CAL-B (10 mg/mL), methyl shikimate (0.1 mmol), divinyl adipate (0.4 mmol), organic solvent (2 mL), 30 °C, 24 h.
[a] Determined by HPLC.[19]
[b] Percentage of regioselectivity at C-4 and C-5.
[c] Yields of products at C-3 were observed very low (<1%).

Table 3. Chemical shifts of ^{13}C NMR of methyl shikimate (DMSO-d_6) and vinyl ester derivatives ($CDCl_3$)

Carbon	**1**	**7a**	**7b**	**7c**	**8a**	**8b**	**8c**
1	127.8	130.0	130.0	129.8	130.1	128.2	129.4
2	140.3	135.9	135.9	136.1	136.0	134.7	135.6
3	67.3	66.2	66.1	66.1	64.8	64.1	64.2
4	70.5	69.8	69.1 1	69.5	74.4	72.9	73.6
5	65.9	69.8	69.1	69.6	64.8	63.6	64.2
6	30.1	28.6	28.5	28.9	31.5	30.7	31.2
7	167.2	166.3	166.5	166.4	166.6	166.9	166.7
8	52.1	52.1	52.1	52.1	52.1	52.2	52.1
$-CH_2-$		34.0	34.2	34.3	33.9	34.0	33.9
		33.4	33.7	33.9	33.3	34.0	33.6
		24.2	28.5	28.9	24.2	28.4	28.6
		23.8	28.5	28.9	23.6	28.4	28.6
			24.6	28.9		24.6	28.6
			24.3	28.3		24.3	28.5
				24.8			24.6
				24.5			24.4
$-C=O$		173.0	173.6	173.8	173.1	173.1	173.1
		170.5	170.8	170.9	170.8	170.9	170.9
$-CH=CH_2$		141.1	141.1	141.1	141.0	141.0	141.1
		97.8	97.6	97.5	98.0	98.4	98.1

The acylating agents also affect the catalytic efficiency of enzyme. Among three divinyl alkyl-α,ω-dicarboxylates, the reaction time of divinyl sebacate is longer than that of divinyl adipate and divinyl suberate if similar high yields are required (Table 1). To be mentioned is that CAL-B and MML show good stability and activity in organic solvents, even when used three times, without a noticeable loss of activity.

The regioselective results were confirmed by ^{13}C NMR spectra as summarized in Table 3. According to the general strategy described by Yoshimoto et al., acylation of a hydroxyl group would lead to the O-acylation carbon downfield shift, while the adjacent carbon upfield shift in ^{13}C NMR.[20] Characterization of the products (**7a**–**7c**) by ^{13}C NMR revealed that vinyl esters of methyl shikimate are substituted at C-5 position and the products (**8a**–**8c**) are substituted at C-4 position. The ^{1}H NMR spectra also provided the substitutional information at different hydroxyl positions.

In summary, a convenient method controllable regioselective enzymatic synthesis for polymerizable derivatives of methyl shikimate was developed in this letter. Excellent regioselectivity of MML was shown toward the C-5 position by using divinyl alkyl-α,ω-dicarboxylates in acetone, THF, and dioxane. In the case of CAL-B, high yields and regioselectivity were achieved in acetone. Additionally, polar solvents such as *tert*-butyl-alcohol, THF, acetone, and dioxane seemed to be suitable for this reaction. These polymerizable derivatives of methyl shikimate obtained have been expected to be a potential new application in natural products and pharmaceutical chemistry.

Acknowledgments

Authors gratefully acknowledge the National Natural Science Foundation of China (Nos. 20702034, 20725206) and Youth Scientific Fund of Sichuan University for financial support.

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17. Ten commercial enzymes: (1) lipase acrylic resin from *Candida antarctica*, (2) lipozyme®, immobilized lipase from *Mucor miehei*, (3) lipase from porcine pancreas, (4) lipase from *Candida cyclindracea*, (5) lipase type VII from *Candida rugose*, (6) amino lipase from *Mucor javanicus*, (7) lipase AY30, (8) protease from *Bacillus species*, (9) protease from *Rhizopus* sp., and (10) protease from *Bacillus licheniformis*.
18. ^{1}H NMR of **7a** ($CDCl_3$, δ, ppm): 7.28 (dd, 1H, —CH=, J = 14.0, 6.0 Hz), 6.89 (m, 1H, H_2), 5.22 (m, 1H, H_5), 4.89 (d, 1H, CH_2=, J = 14.0 Hz), 4.58 (d, 1H, CH_2=, J = 6.4 Hz), 4.45 (m, 1H, H_3), 3.87 (dd, 1H, H_4, J = 4.2, 8.2 Hz), 3.77 (s, 3H, —OCH_3), 2.92 (dd, 1H, H_6, J = 18.2, 5.2 Hz), 2.41 (m, 4H, 2-CH_2—CO—), 2.31 (dd, 1H, H_6, J = 18.2, 6.4 Hz), 1.70 (m, 4H, —CH_2—). HR-MS of **7a** (*m*/*z*): 365.1213 $[M_1+Na]^+$, M_1 corresponding exactly to 5-*O*-vinyladipate-methyl shikimate's molecular weight. ^{1}H NMR of **8a** ($CDCl_3$, δ, ppm): 7.28 (dd, 1H, —CH=, J = 14.0, 6.4 Hz), 6.86 (m, 1H, H_2), 4.96 (dd, 1H, H_4, J = 4.0, 8.0 Hz), 4.89 (d, 1H, CH_2=, J = 14 Hz), 4.66 (m, 1H, H_3), 4.59 (d, 1H, CH_2=, J = 6.0 Hz), 4.25 (m, 1H, H_5), 3.78 (s, 3H, —OCH_3), 2.84 (dd, 1H, H_6, J = 18.0, 5.0 Hz), 2.44 (m, 4H, 2-CH_2—CO—), 2.35 (dd, 1H, H_6, J = 18.6, 6.4 Hz), 1.71 (m, 4H, —CH_2—). HR-MS of **8a** (*m*/*z*): 365.1213 $[M_2+Na]^+$, M_2 corresponding exactly to 4-*O*-vinyladipate-methyl shikimate's molecular weight. HR-MS of **7b** (*m*/*z*): 393.1533 $[M_3+Na]^+$, M_3 corresponding exactly to 5-*O*-vinylsuberate-methyl shikimate's molecular weight. HR-MS of **7c** (*m*/*z*): 421.1839 $[M_4+Na]^+$, M_4 corresponding exactly to 5-*O*-vinylsebacate-methyl shikimate's molecular weight.
19. HPLC analysis: samples were dissolved in methanol and analyzed by HPLC using a Kromasil ODS-1 (5 μm, 4.6 × 250 mm) in Shimadzu 2010A system, eluted with methanol/water (70:30, v/v) at 0.8 mL/min, and UV detection was carried out at 213 nm.
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