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Two lipase-catalyzed sequential synthesis of drug derivatives in organic media

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ABSTRACT

The study first reported the efficient lipase catalysis of aza-Markovnikov addition of *N*-heterocycles to vinyl esters in organic media. After screening the enzyme sources and organic solvents, the yield was up to 82.6% and the reaction rate increased more than 600-folds under the catalysis of Amino lipase M from *Mucor javanicus* in DMSO. Some control experiments were designed to demonstrate the catalytic specificity of lipase. A new strategy for the enzymatic synthesis of drug derivatives was developed by combining aza-Markovnikov addition with acylation procedure involving divinyl esters as linkers. A series of drug derivatives containing *N*-heterocycles were successfully obtained. The new activity of lipase expands the application of biocatalyst and provides a useful avenue to synthesize drug derivatives.

1. Introduction

Enzymes can be used as efficient catalysts in organic synthesis [1–3]. However, the highly specific property of enzymes leads to searching for various candidates to promote different reactions. Recently, plenty of studies have clearly demonstrated that enzymes have catalytic promiscuity which exhibits their selectivity for non-natural functional groups. Therefore, their splendid applications in synthetic chemistry have been largely expanded. Desaturase exhibited a new activity in oxidation of the chiral 9-fluorinated substrates [4]. *N*-Acylamino acid racemase acted as *O*-succinylbenzoate synthase [5]. Subtilisin catalyzed not only hydrolysis of a sulfonamide S—N bond in an *N*-acyl sulfonamide, but also Michael addition of *N*-nucleophiles to acrylates [6–10]. Acylase was proved to promote aza-Markovnikov reaction [11–13]. In the further study, it also showed a remarkable catalytic ability for Michael addition [14,15]. As a commercially available and widely used enzyme, lipase shows its great potential application as well. Some lipases can be used as carboxylic acid esterases, thioesterases, peptidases, dehalogenases, epoxide hydrolases and halo peroxidases, *etc.* [16,17]. Kitazume et al. used hydrolytic enzymes to catalyze the Michael addition of fluorine-containing compounds in aqueous phase. Therefore, efforts to develop lipase-catalyzed addition in organic media have become to increase in popularity [18]. Berglund used an engineered mutant of CAL-B to catalyze Aldol reaction and Michael addition [19–21]. Our group and Gotor both found that wild-type lipase was also capable of catalyzing Michael type addition in non-aqueous media [22,23]. These promiscuities intrigue the great interest in studying new catalytic activities and provide a useful avenue to construct complicated backbones [24,25].

In view of the important role of lipase in biosynthesis, the exploration of their novel activities attracted our interest. In this paper, we discovered that lipase was able to catalyze aza-Markovnikov addition of *N*-heterocycles to vinyl esters in organic media as Scheme 1. The best result was achieved when Amino lipase M from *Mucor javanicus* (MJML) was used as biocatalyst. Based on it, a promising protocol for synthesis of drug derivatives was established in which enzymatic aza-Markovnikov addition was combined with a lipase-catalyzed acylation procedure in non-aqueous media. Divinyl esters were chosen to link *N*-heterocycles and drugs. The sequential aza-Markovnikov addition and acylation were under the catalysis of MJML and CAL-B, respectively. A series of drug derivatives containing *N*-heterocycles were prepared with considerable yields.

2. Materials and methods

2.1. Materials

Immobilized lipase from *Mucor miehei* (Lipozyme®), lipase from *M. javanicus* (MJL), lipase from hog pancreas (HPL) and lipase from *Candida cylindracea* (CCL) were purchased from Fluka. Lipase from porcine pancreas (PPL), lipase from *Candida rugose* (CRL) and lipase acrylic resin from *Candida antarctica* (CAL-B) were purchased

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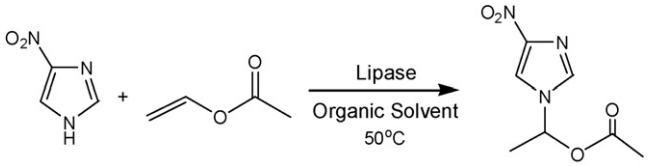


Scheme 1. Lipase-catalyzed aza-Markovnikov addition of 4-nitro-imidazole to vinyl acetate in organic solvents.

from Sigma. Amino lipase M from *M. javanicus* (MJML) was purchased from Aldrich. Bovine Serum Albumin (BSA) was purchased from Sino-American Biotechnology Co. Solvents were dried over 3 Å molecular sieves for 24 h prior to use. All other chemicals were of the highest purity commercially available.

2.2. Analytical methods

The reactions were monitored by TLC on silica gel plates with petroleum ether/ethyl acetate as eluent. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX-500 MHz spectrometer, using DMSO-d_6 as a solvent and TMS as an internal reference. Analytical HPLC was performed using a SHIMADZU LC-10AVP HPLC with a UV detector. IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Mass spectra were obtained by electrospray ionization (ESI) on a Bruker esquire 3000 plus mass spectrometer.

2.3. General procedure for the enzymatic aza-Markovnikov addition of N-heterocycles to vinyl esters

Enzymatic aza-Markovnikov addition of *N*-heterocycles to vinyl esters was illustrated as Scheme 2. A suspension of **1a–c** (1 mmol) and 25 mg enzyme in 2 ml DMSO was incubated at 50 °C and shaken at 200 rpm for 5 min. Then, 3 mmol vinyl ester (**2a–b**) was added in order to initiate the reaction. The enzyme was filtered off to terminate the reaction and washed with MeOH (3–5 ml). Solvent was evaporated under vacuum to dryness. The products were separated by silica gel column chromatography with an eluent consisting petroleum ether/ethyl acetate. Product-contained fractions were combined, concentrated, and dried.

2.3.1. 1-(1-(4-Nitro-imidazole))-ethyl vinyl adipate (**3a**)

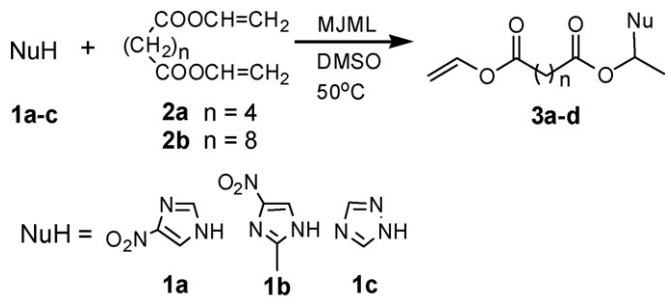
Yellow oil; yield 83%; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 8.66 (s, 1H, N—CH—N), 8.10 (s, 1H, N—CH—C), 7.20 (m, 1H, O—CH—CH_2), 6.79 (q, 1H, J = 6.19 Hz, N—CH—O–C—O), 4.89, 4.64 (m, 2H, O—CH—CH_2), 2.42–2.36 (m, 4H, O—C—CH_2), 1.78 (d, 3H, J = 6.20 Hz,—CH—CH_3), 1.52 (t, 4H, O—C—CH_2—CH_2—CH_2). ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 172.00, 170.65, 147.80, 141.66, 137.08, 119.94, 98.46, 77.29, 33.19, 33.02, 23.76, 23.70, 20.15; IR (neat, cm^{-1}): 1750, 1658, 1514; MS(ESI): $[M + Na]^+$ = 306.0.

2.3.2. 1-(1-(4-Nitro-imidazole))-ethyl vinyl sebacate (**3b**)

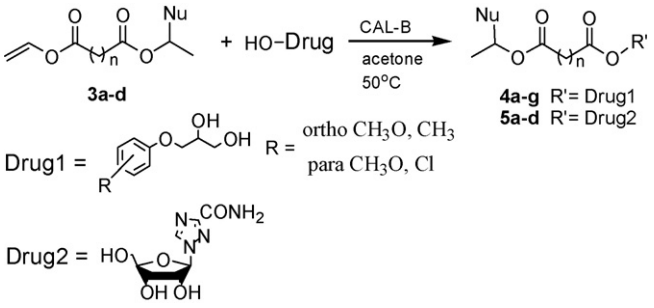
Yellow oil; yield 83%; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 7.95 (d, 1H, N—CH—N), 7.70 (s, 1H, N—CH—C), 7.28 (m, 1H, O—CH—CH_2), 6.72 (q, 1H, J = 6.19 Hz, N—CH—O–C—O), 4.88 (q, 1 H), 4.56 (q, 1H, O—CH—CH_2), 2.35 (m, 4H, O—C—CH_2), 1.85 (d, 3H, J = 6.20 Hz,—CH—CH_3), 1.62 (m, 4H, O—C—CH_2—CH_2—CH_2),1.30 (m, 8 H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 172.36, 171.04, 147.61, 141.36, 135.50, 117.26, 97.70, 75.95, 34.04, 33.96, 29.10, 29.05, 28.97, 24.66, 24.58, 20.40; IR (neat, cm^{-1}): 1748, 1654, 1516; MS(ESI): $[M + Na]^+$ = 390.1.

2.3.3. 1-(1-(2-Methyl-4-nitro-imidazole))-ethyl vinyl sebacate (**3c**)

Yellow oil; yield 12%; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 7.85 (s, 1H, N—CH—C), 7.27 (m, 1 H), 6.65 (d, 1H, J = 5.85 Hz, N—CH—O—C—O), 4.88 (q, 1 H), 4.56 (q, 1H, O—CH—CH_2), 2.55 (s, 3H, N—C—CH_3), 2.34 (m 4 H), 1.77 (d, 3H, J = 5.77 Hz, —CH—CH_3), 1.62 (m, 4 H), 1.28 (s, 8 H); ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 172.04, 171.06, 144.90, 141.42, 133.02, 116.20, 97.74, 75.11, 34.10, 34.00,, 29.17, 29.12, 29.06, 24.78, 24.72, 24.70, 21.38, 13.57; IR (neat, cm^{-1}): 3147, 2942, 1745, 1645, 1542, 1280; MS(ESI): $[M + Na]^+$ = 404.1.



Scheme 2. Synthesis of *N*-heterocycles derivatives catalyzed by MJML.



Scheme 3. Synthesis of drug derivatives catalyzed by CAL-B.

2.3.4. 1-(1-Triazole)-ethyl vinyl sebacate (**3d**)

Yellow oil; yield 58%; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm): 8.32 (s, 1H, N—N—CH—N), 7.96 (s, 1H, N—N—CH—N—), 7.28 (m, 1H, O—CH—CH_2), 6.87 (q, 1H, J = 6.23 Hz, N—CH—O—C—O), 4.86 (q, 1H, O—CH—CH_2), 4.56 (q, 1H, O—CH—CH_2), 2.32 (m, 4H, O—C—CH_2—CH_2—), 1.87 (d, 3H, J = 6.25 Hz, —CH—CH_3), 1.59 (m, 4 H), 1.28 (m, 8 H). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 172.73, 171.04, 152.33, 144.20, 141.42, 97.70, 76.62, 34.13, 33.10, 29.15, 29.10, 29.00, 24.73, 24.70, 19.26; IR (neat, cm^{-1}): 2930, 1748, 1647, 1145; MS(ESI): $[M + Na]^+$ = 290.0.

2.4. General procedure for the enzymatic acylation of drugs

Enzymatic acylation of drugs was illustrated as Scheme 3. The reaction was initiated by adding 10 mg CAL-B to 2 ml acetone containing 0.5 mmol drugs, 1.5 mmol vinyl esters (**3a–d**) respectively. The suspension was kept at 50 °C and shaken at 200 rpm. The reaction was terminated by filtering off the enzyme, and the filtrate was concentrated under reduced pressure. The products were separated by silica gel column chromatography with an eluent consisting petroleum ether/ethyl acetate or ethyl acetate/methanol/water. Product-contained fractions were combined, concentrated, and dried.

2.4.1. 1′-O-[1-(1-(4-Nitro-imidazole))-ethyl vinyl adipate]-guaifenesin (**4a**)

Yellow oil; yield 20%; ^{1}H NMR ($CDCl_3$, δ, ppm): 7.97 (s, 1H, CH—N), 7.70 (s, 1H, N—CH—N), 6.94 (m, 4H, CH—C), 6.69 (q, 1H, J = 6.25 Hz, O—CH—N), 4.27 (m, 1H, CH—OH), 4.22 (m, 2H, CH_2—O), 4.09 (m, 1H, CH_2—O), 4.02 (m, 1H, CH_2—O), 3.85 (s, 3H, O—CH_3), 3.61 (w, 1H, —OH), 2.36 (m, 4H, CH_2—C—O), 1.84 (d, 3H, J = 6.25 Hz, CH_3—CH), 1.63 (m, 4H, CH_2—CH_2); ^{13}C NMR ($CDCl_3$, δ, ppm): 173.29, 171.91, 150.05, 148.12, 135.50, 122.63, 121.18, 117.33, 115.57, 112.26, 76.08, 71.46, 68.51, 65.42, 56.01, 33.799, 33.65, 24.15, 23.96, 20.33; IR (neat, cm^{-1}): 3478, 3132, 2944, 2838, 1732, 749; MS(ESI): $[M + Na]^+$ = 488.1.

2.4.2. 1′-O-[1-(1-(4-Nitro-imidazole))-ethyl vinyl sebacate]-guaifenesin (**4b**)

Yellow oil; yield 37%; ^{1}H NMR ($CDCl_3$, δ, ppm): 7.96 (s, 1H, CH—N), 7.71 (s, 1H, N—CH—N), 6.93 (m, 4H, CH—C), 6.71 (q, 1H, J = 6.29 Hz, O—CH—N), 4.27 (m, 1H, CH—OH), 4.23 (m, 2H, CH_2—O), 4.08 (m, 1H, CH_2—O), 4.02 (m, 1H, CH_2—O), 3.84 (s, 3H, O—CH_3), 2.33 (m, 4H, CH_2—C—O), 1.84 (d, 3H, J = 6.27 Hz, CH_3—CH), 1.58 (m, 4H, CH_2—CH_2), 1.27 (m, 8H, CH_2—CH_2); ^{13}C NMR ($CDCl_3$, δ, ppm): 173.98, 172.31, 150.05, 148.11, 135.48, 122.56, 121.20, 117.28, 115.58, 112.24, 75.93, 71.46, 68.53, 65.16, 55.98, 34.16, 33.89, 29.16, 29.08, 29.04, 29.03, 24.94, 24.89, 20.31; IR (neat, cm^{-1}): 3467, 3131, 2931, 2856, 1737, 747; MS(ESI): $[M + Na]^+$ = 544.1.

2.4.3. 1′-O-[1-(1-(2-Methyl-4-nitro-imidazole))-ethyl vinyl sebacate]-guaifenesin (**4c**)

Yellow oil; yield 57%; ^{1}H NMR ($CDCl_3$, δ, ppm): 7.86 (s, 1H, CH—N), 6.94 (m, 4H, CH—C), 6.64 (q, 1H, J = 6.235 Hz, O—CH—N), 4.26 (m, 1H, CH—OH), 4.23 (m, 2H, CH_2—O), 4.06 (m, 2H, CH_2—O), 3.85 (s, 3H, O—CH_3), 2.55 (s, 3H, CH_3—C), 2.33 (m, 4H, CH_2—C—O), 1.76 (d, 3H, J = 6.24 Hz, CH_3—CH), 1.58 (m, 4H, CH_2—CH_2), 1.27 (m, 8H, CH_2—CH_2); ^{13}C NMR ($CDCl_3$, δ, ppm): 174.04, 172.02, 150.15, 148.15, 147.01, 144.89, 122.65, 121.26, 116.24, 115.63, 112.27, 75.10, 71.58, 68.65, 65.21, 56.02, 34.23, 33.93, 29.13, 29.11, 29.07, 28.99, 24.96, 24.64, 21,24, 13.50; IR (neat, cm^{-1}): 3460, 3142, 2931, 2857, 1733, 747; MS(ESI): $[M + Na]^+$ = 558.1.

2.4.4. 1′-O-[1-(1-Triazole)-ethyl vinyl sebacate]-guaifenesin (**4d**)

Yellow oil; yield 93%; ^{1}H NMR ($CDCl_3$, δ, ppm): 8.32 (s, 1H, N—CH—N), 7.97 (s, 1H, N—CH—N), 6.95 (m, 4H, CH—C), 6.86 (d, 1H, J = 6.25 Hz, O—CH—N), 4.27 (m, 1H, CH—OH), 4.24 (m, 2H, CH_2—O), 4.09 (m, 1H, CH_2—O), 4.02 (m, 1H, CH_2—O), 3.86 (s, 3H, O—CH_3), 2.32 (m, 4H, CH_2—C—O), 1.87 (d, 3H, J = 6.245 Hz, CH_3—CH), 1.58 (m, 4H, CH_2—CH_2), 1.27 (m, 8H, CH_2—CH_2); ^{13}C NMR ($CDCl_3$, δ, ppm): 174.06, 172.73, 152.21, 149.98, 148.13, 144.18, 122.52, 121.25, 115.35, 112.17, 76.58, 71.44,