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A single-enzyme, two-step, one-pot synthesis of *N*-substituted imidazole derivatives containing a glucose branch *via* combined acylation/Michael addition reaction†

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Combined regioselective acylation/Michael addition reaction catalyzed by alkaline protease from *Bacillus subtilis* in anhydrous pyridine for synthesis of *N*-substituted imidazole derivatives containing a glucose branch *via* a novel single-enzyme, two-step, one-pot procedure is reported.

Combined catalytic, multistep, one-pot conversions, based on the principle of metabolic pathways *in vitro*,[1] offer potential advantages for the preparation of many compounds *i.e.* saving the effort of isolating intermediates and avoiding the accumulation of reactive and/or unstable intermediates. In particular, multienzyme cascade reactions in one-pot synthesis have led to the development of a variety of elegant and effective synthetic methods.[2] Wong demonstrated the L-fructose synthesis starting from glycerol using coupled enzymatic systems with galactose oxidase, catalase, rhamnulose-1-phosphate aldolase, and acid phosphatase.[3] Multienzyme cascades have also been used in the synthesis of complex carbohydrate derivatives,[4] nonnatural carbohydrates,[5] cephalexin,[6] cefazolin[7] and riboflavin,[8] as well as in the synthesis of sialylated Thomsen–Friedenreich antigen determinant.[9] However, a multienzyme system usually requires specific conditions for every step, *e.g.* pH and temperature *etc.*, and this needs a laborious and precise manipulation process,[5–6] even the other enzyme sometimes can't tolerate the specific conditions in one pot. In addition, there may be some possible undesired interferences among multi-enzymes or inhibitory effects of starting reagents on the succeeding enzymatic reactions. Single-enzyme multistep conversions can overcome these obstacles. Nevertheless the enzyme which can catalyze multistep reactions is very scarce. Recent advances in 'protein engineering' have yielded bifunctional fusion enzymes by expressing two enzymatic activities as a single protein.[10] Single-lipase-mediated multistep techniques have been used in Novozym® 435-catalyzed deprotection, acetylation, epoxidation[11] and lipase LIP-catalyzed domino kinetic resolution/intramolecular Diels–Alder reaction.[12] To the best of our knowledge, single-protease-mediated two-step acylation/Michael addition reaction in one pot hasn't been demonstrated.

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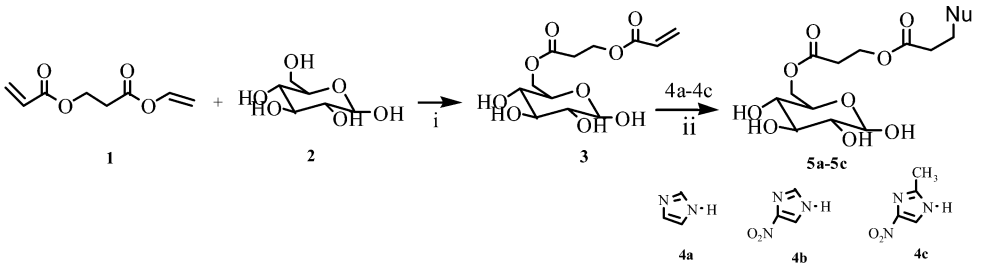
The Michael addition is among the most useful carbon–carbon bond forming reactions and has wide synthetic application in organic synthesis.[13] It has received great attention in recent years, especially in view of the advantages in terms of selectivity and environmental friendliness that can be achieved by using new catalysts such as guanidinium,[14] RNA,[15] antibody,[16] baker's yeast and lipases[17] instead of the traditional basic catalysts. We have already reported that alkaline protease from *Bacillus subtilis* is an efficient catalyst for Michael addition of pyrimidine and imidazole,[18] as well as in the regioselective acylation of carbohydrates.[19] Therefore, we planned a novel reaction system based on the idea of combining the protease-catalyzed acylation/Michael addition into a single-enzyme, two-step, one-pot synthesis. We herein report the successful application of this concept of a one-pot procedure, comprising a single-protease-mediated two-step cascade glucose-selective-acylation and imidazole derivatives-Michael addition to afford novel *N*-substituted imidazole derivatives containing a D-glucose branch (Scheme 1) which may possess potential anticancer activities.[20]

Our initial strategy was to accomplish the total synthesis of **5a–5c** in several separate steps. The synthetic route involves vinyl 3-propenoyloxy propionate **1**, which was obtained from acrylic acid and vinyl acetate, as the starting compound. The acylation of **1** with D-glucose **2** catalyzed by alkaline protease from *Bacillus subtilis* in pyridine at 50 °C for 36 h gave an anomeric mixture of 6-*O*-(β-propenoyloxy-propionyl)-D-glucose **3** (α : β = 40 : 60) in 83% isolated yield. The succeeding Michael addition reaction of **4a–4c** to **3** was carried out under the same conditions as those of the acylation reaction using fresh protease. The reaction was completed after 20 h. The expected Michael adduct **5a–5c** was thus obtained, after purification, in isolated yield from 69%–80%.

All novel compounds **3**, **5a–5c** were characterized by IR, ^{1}H NMR and ^{13}C NMR‡. These data were in agreement with the desired structure.

Next, we carried out the single-protease-mediated two-step cascade reaction in the reverse sequence. The Michael addition reaction of **4b** to **1** was carried out under the aforementioned conditions. Michael adduct 3-(4-nitroimidazol-1-yl)propionic acid 2-vinyloxycarbonyl-ethyl ester **6b** (Scheme 2) was obtained in 44% isolated yield. The succeeding selective acylation of D-glucose **2**



Scheme 1 *Reagents and conditions*: (i) alkaline protease from *Bacillus subtilis*, pyridine, 50 °C, 36 h; (ii) alkaline protease from *Bacillus subtilis*, pyridine, 50 °C, 20 h.

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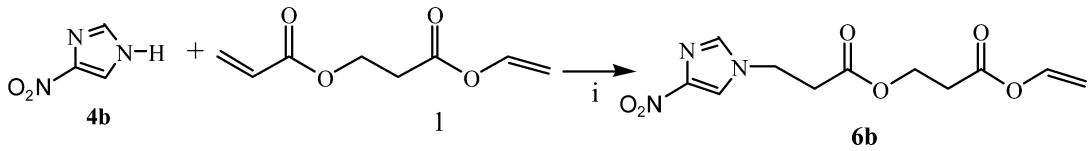

Scheme 2 *Reagents and conditions*: (i) alkaline protease from *Bacillus subtilis*, pyridine, 50 °C.

with **6b** using fresh protease afforded the final product **5b** in 34% total yield.

In order to avoid the intermediate purification and answer the interesting questions: whether the alkaline protease can simultaneously catalyze acylation and Michael addition reaction, namely, is there the undesired interference and competition between the two catalytic activities of the protease, and which one activity is higher than the other in absolutely the same conditions? one-pot syntheses of **5b** were performed. We first carried out the one-pot synthesis according to the following process. During the synthesis of compound **3**, after the disappearance of **1** (monitored by TLC), without purification, **4b** was directly added to the mixture; Michael addition reaction of **4b** to **3** was achieved under the aforementioned conditions, the expected Michael adduct **5b** was also efficiently obtained in 68% total isolated yield. Then, we performed the cascade reactions in the complete one-pot manner. Three components, **1**, **2** and **4b**, were simultaneously added to the reaction system. The process of the one-pot reaction catalyzed by the protease was monitored by TLC. After 2 h, **3** appeared, while Michael adduct **6b** between **1** and **4b** appeared after 6 hours; a quicker increase of **3** than **6b** was observed. The final adduct **5b** appeared 20 h later. After 70 h, **1**, **3** and Michael adduct **6b** all disappeared. The total isolated yield of **5b** was 61%. The results show that the catalytic activity for acylation of the protease is higher than the activity for Michael addition in pyridine. It also confirmed that the one-pot synthesis of combined acylation/Michael addition can be efficiently performed by the protease.

Neither acylation of **2** and **1** nor Michael addition reaction of **4a–4c** and **3** in pyridine occurred in the absence of enzyme even after 14 days. A study of the kinetics revealed the alkaline protease with the ability to catalyze the second step Michael-addition at rate accelerations k_{cat}/k_{uncat} over background of up to 2.3×10^5-fold.

The combination of bi-catalytic activities of alkaline protease from *Bacillus subtilis* in non-aqueous media for acylation and Michael addition reaction in one pot provides an attractive procedure for imidazole–sugar conjugates synthesis without intermediate recovery steps, use of protective groups or reagents. The first successful demonstration of a single-protease, two-step, one-pot synthesis *via* combined acylation/Michael addition reaction opens up opportunities for further transformations of a finely designed process that starts from multifunctional substrates and a wide variety of Michael donors. A detailed evaluation of the novel reaction system is currently in progress in our laboratory.

Notes and references

‡ Selected data for **5a**: IR (cm^{-1}): 3346 (ν_{O-H}), 1733 ($\nu_{C=O}$), 1577 ($\nu_{C=C}$), 1515 ($\nu_{C=N}$), 2924 (ν_{-CH_2-}). ^{1}H NMR (500 MHz, D_2O, δ, ppm): 7.97 (s, 1H, $C_{(2)}$–H, imidazole), 7.19 (s, 1H, $C_{(4/5)}$–H, imidazole), 7.06 (s, 1H, $C_{(4/5)}$–H, imidazole), 5.09 (d, 0.4H, J = 3.73 Hz, H-1 of α-D-glucose), 4.54 (d, 0.6H, J = 7.98 Hz, H-1 of β-D-glucose), 4.33–4.15 (m, 6H, H-6, 6′ of β-D-glucose, $-CH_2-O-$, $-CH_2-N$, H-6, 6′ of α-D-glucose), 3.14 (t, 0.6H, J = 8.49 Hz, H-2 of β-D-glucose), −3.32 (other αH or βH of D-glucose), 2.85, 2.65 (m, m, 4H, O=C–CH_2, O=C–CH_2). ^{13}C NMR (500 MHz, D_2O, δ, ppm): 172.97, 172.57 (C=O), 136.95 (C-2, imidazole), 124.88 (C-5, imidazole), 120.63 (C-4, imidazole), 96.05 (C1 of β-D-glucose), 92.17 (C1 of α-D-glucose), 75.54 (C3 of β-D-glucose), 74.06 (C2 of β-D-glucose), 73.31 (C5 of β-D-glucose), 72.59 (C3 of α-D-glucose), 71.44 (C2 of α-D-glucose), 69.57 (C4 of α-D-glucose), 69.47 (C4 of β-D-glucose), 69.13 (C5 of α-D-glucose), 63.70, 63.68 (C6α,β of D-glucose), 60.72 ($-CH_2-O$), 43.11 ($-CH_2-N$), 34.82, 33.14 (CH_2 of O=C–CH_2).

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