Supplementary Material (ESI) for Chemical Communications

Promiscuous zinc-dependent acylase-mediated carbon-carbon bond formation in organic media

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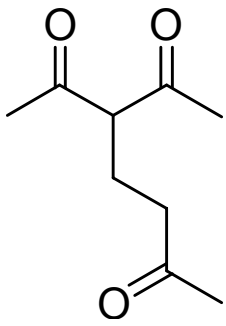
1. General analytical apparatuses and methods:

TLC (silica gel; petroleum–ethyl acetate, 3:1) was used to monitor the reaction. The structure of the adducts and position of addition was confirmed by ^{1}H NMR and ^{13}C NMR (Bruker AMX-500 MHz) using $CDCl_3$ as solvent and chemical shifts are expressed in ppm with reference to Me_4Si. The progress curve of the enzymatic Michael addition were analyzed by GC with SE-54 capillary column and FID detection. Oven temperature: from 60 °C to 200 °C, rate of heating 20 °C $min.^{-1}$. 1,3-dicarbonyl compounds and methyl vinyl ketone were distilled before use. Analytical grade solvents were dried by molecule sieve before use.

2. Typical experimental procedure

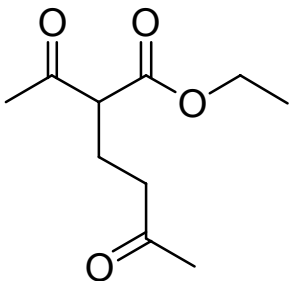
In a conical flask containing 10 mg DA, a solution of internal standard (dodecane) and methyl vinyl ketone (2 mmol) in 1 mL 2-Methyl-2-butanol was incubated at 50 °C and 250 r.p.m. (orbitally shaken) for 20 minutes. Then, active methylene compound (1 mmol) was added in order to initiate the reaction. After indicated time, the enzyme was filtered off to terminate the reaction and washed with MeOH (3-5 mL). Solvent was evaporated under vacuum to dryness. The crude residue was purified by flash column chromatography on silica gel using petroleum/ethyl mixtures. Product-contained fractions were combined, concentrated, and dried to give Michael adduct. All the compounds were spectroscopically characterized (IR, ^{1}H NMR, ^{13}C NMR and MS) and analytically compared (GC) with true samples prepared by conventional methods. The reactions were run at least twice.

3-Acetyl-heptane-2,6-dione



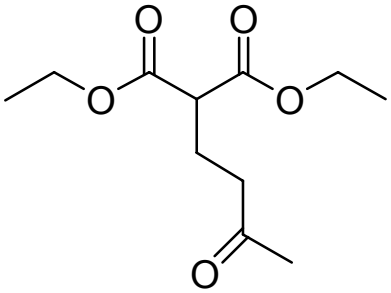
^{1}H-NMR (CDCl3, δ, ppm): 3.68 (t, 1 H, J=6.93, C(3)-H), 2.45 (t, 2 H, J=7.00, C(5)-H), 2.20 (s, 6 H, $CH_3COCHCOCH_3$), 2.13 (s, 3 H, C(7)-H), 2.08 (m, 2 H, C(4)-H). ^{13}C NMR (CDCl3, δ, ppm): 207.7, 204.3, 67.1, 44.2, 40.7, 29.5, 21.6. IR (cm^{-1}): 1716 (-C=O), 1358 (-CH_2), 1167 (C-O).

2-Acetyl-5-oxo-hexanoic acid ethyl ester



^{1}H-NMR (CDCl3, δ, ppm): 4.20 (q, 2 H, -OC$\underline{H}_2$-) , 3.50 (t, 1 H, J=7.1, O=CC$\underline{H}$C=O), 2.50 (t, 2 H, J=7.00, $CH_3COC\underline{H}_2$-), 2.20 (s, 3 H, C$\underline{H}_3$COCH-), 2.13 (s, 3 H, C$\underline{H}_3COCH_2CH_2$-), 2.08 (m, 2 H, $CH_3COCHC\underline{H}_2CH_2$-), 1.28 (t, 3 H, -$OCH_2C\underline{H}_3$). ^{13}C NMR (CDCl3, δ, ppm): 207.6, 203.0, 169.6, 61.6, 58.4, 40.6, 30.0, 29.1, 21.8, 14.2. IR (cm^{-1}): 1739 (O=C-O-), 1715 (-C=O), 1360 (-CH_2), 1153 (C-O).

2-(3-Oxo-butyl)-malonic acid diethyl ester



^{1}H-NMR (CDCl3, δ, ppm): 4.20 (m, 4 H, -OC$\underline{H}_2$-), 3.39 (t, 1 H, J=7.3, O=CC$\underline{H}$C=O), 2.54 (t, 2 H, J=7.30, $CH_3COC\underline{H}_2$-), 2.16 (m, 2 H, $CH_3COCHC\underline{H}_2$-), 2.13 (s, 3 H, C$\underline{H}_3COCH_2CH_2$-), 1.27 (m, 6 H, -$OCH_2C\underline{H}_3$). ^{13}C NMR (CDCl3, δ, ppm): 207.4, 169.4, 61.7, 50.9, 40.7, 30.1, 22.7, 14.3. IR (cm^{-1}): 1727 (O=C-O-), 1367 (-CH_2), 1186 (C-O).