Supplementary Information (SI) for Green Chemistry.
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Scheme S1 The terminal C-H bond hydroxylation of dodecane catalyzed by AlkB. AlkB is a non-heme diiron monooxygenase. During the catalytic cycle, the diiron center activates molecular oxygen (O_2). One oxygen atom is inserted into the terminal C-H bond of the alkane substrate. Concurrently, the second oxygen atom acts as the terminal acceptor for the hydrogen atoms—specifically, the hydride equivalent derived from NADH (transferred via the PdR-AlkG electron cascade) and a proton—to ultimately generate a molecule of water (H_2O). Thus, the hydrogen atoms removed during this oxidation process are transferred to form H_2O.[1,2]

Scheme S2 The scheme of multi-enzymatic conversion from dodecane to dodecanoic acid coupled with NADH regeneration.

Scheme S3 ω-hydroxylation of 12-hydroxydodecanoic acid catalyzed by $CYP153A_{Maq}$. $CYP153A_{Maq}$ is a cytochrome P450 monooxygenase. In its catalytic cycle, the heme iron center activates O_2. One oxygen atom is incorporated into the ω-position of dodecanoic acid to form the hydroxyl group. The second oxygen atom serves as the terminal acceptor for the hydrogen atoms (the electron/hydride equivalents donated by NADH via the PdR-Pdx chain, along with a proton), yielding a molecule of H_2O.[3]

Scheme S4 A multi-enzyme cascade conversion scheme for the conversion of dodecanoic acid to dodecanedioic acid ingeniously integrates an internal NADH cycle regeneration system.

Fig. S1 SDS-PAGE analysis of purified enzymes used in this study. (a) Lane M: marker; Lane 1: AlkB (46.9 kDa); Lane 2: EcALDH (54.2 kDa); Lane 3: $AcCO_6$ (60.9 kDa); Lane 4: $CYP153A_{Maq}$ (55.1 kDa); Lane 5: CatA (55.9 kDa); Lane 6: PdR (46.6 kDa); Lane 7: Pdx (12.6 kDa); Lane 8: AlkG (20.1 kDa).

Fig. S2 CO difference spectra of purified $CYP153A_{Maq}$. Black curve: The CO statured $CYP153A_{Maq}$ sample; Red curve: Added $Na_2S_2O_4$ to the CO statured $CYP153A_{Maq}$ sample; Blue curve: CO difference spectra.

Fig. S3 GC analysis of the formation of dodecanol and dodecanal catalyzed by AlkB. The reactions were performed in a 7 mL sealed vial with magnetic string at room temperature. The 500 µL reaction mixture contained 100 mM KPi buffer (pH=8.0), 10 µM (0.06 U/mL) AlkB, 30 µM AlkG, 60 µM PdR (molar ratio of AlkB: AlkG: PdR=1: 3: 6), 4 mM NADH and 0.75% (*v*/*v*) dodecane as substrate. After the reactions were completed, the reaction mixtures were extracted with MTBE containing 0.5 mM n-eicosane as an internal standard.

Fig. S4 GC analysis of the formation of dodecanoic acid catalyzed by EcALDH. The reactions were performed in a 7 mL sealed vial with magnetic string at room temperature. The 500 µL reaction mixture contained 100 mM KPi buffer (pH=8.0), 10 µM (0.20 U/mL) EcALDH, 4 mM NAD^+ and 2 mM dodecanal. After the reactions were completed, the reaction mixtures were extracted with MTBE containing 0.5 mM n-eicosane as an internal standard.

Fig. S5 GC analysis of the formation of 12-hydroxydodecanoic acid catalyzed by $CYP153A_{Maq}$. The reactions were performed in a 7 mL sealed vial with magnetic string at room temperature. The 500 µL reaction mixture contained 100 mM KPi buffer (pH=8.0), 30 mM $MgCl_2$, 10 µM (0.10 U/mL) $CYP153A_{Maq}$, 10 µM PdR, 100 µM Pdx (molar ratio of $CYP153A_{Maq}$: PdR: Pdx=1: 1: 10), 2 mM NADH and 2 mM dodecanoic acid. After the reaction was completed, the reaction mixture was first acidified with 10% (*v*/*v*) hydrochloric acid. Then, MTBE containing 0.5 mM n-eicosane as an internal standard was added for extraction.

Fig. S6 a GC analysis of the formation of dodecanedioic acid catalyzed by $AcCO_6$ and EcALDH. (i) GC analysis of dodecanedioic acid standard. (ii) GC analysis of the negative control (without $AcCO_6$ and EcALDH) using 12-hydroxydodecanoic acid as the substrate. (iii) GC analysis of the conversion from 12-hydroxydodecanoic acid to 12-oxododecanoic acid catalyzed by $AcCO_6$. (ⅳ) GC analysis of the conversion from 12-hydroxydodecanoic acid to dodecanedioic acid catalyzed by $AcCO_6$ and EcALDH. $AcCO_6$-catalyzed reactions were performed in a 7 mL sealed vial with magnetic string at room temperature. The 500 µL reaction mixture contained 100 mM KPi buffer (pH=8.0), 10 µM (0.003 U/mL) $AcCO_6$ and 2 mM 12-hydroxydodecanoic acid. Since 12-oxododecanoic acid standards were unavailable, we indirectly verified the aldehyde dehydrogenase activity of EcALDH by adding 10 µM (0.17 U/mL) EcALDH and 2 mM NAD^+ to the reaction mixture described above, thereby converting the intermediate 12-oxododecanoic acid into dodecanedioic acid. After the reactions were completed, the reaction mixtures were first acidified with

10% (*v*/*v*) hydrochloric acid. Then, MTBE containing 0.5 mM n-eicosane as an internal standard was added for extraction. The derivatization method reported by Slaughter et al.[4] was used for sample processing. **b** When $AcCO_6$ catalyzed the conversion of 12-hydroxydodecanoic acid, a new product peak (labeled P1) was observed (**Figure S6a**). LC-MS analysis confirmed this product to be 12-oxododecanoic acid.

Fig. S7 Optimization of the enzyme ratio for $CYP153A_{Maq}$ to $AcCO_6$. **a** The enzyme concentration of $CYP153A_{Maq}$ was optimized. With the Mg^{2+} concentration fixed at 30 mM, $CYP153A_{Maq}$ was added at varying concentrations. **b** The enzyme ratio of $CYP153A_{Maq}$ to $AcCO_6$ was optimized. With $CYP153A_{Maq}$ concentration fixed at 20 μM (0.19 U/mL), $AcCO_6$ was added at varying concentrations.

Fig. S8 GC calibration curve for the quantification analysis of dodecanol (a), dodecanal (b), 12-hydroxydodecanoic acid (c) , hexanoic acid (d), heptanoic acid (e), octanoic acid (f), nonanoic acid (g) and decanoic acid (h).

Fig. S9 GC calibration curve for the quantification analysis of undecanoic acid (a), dodecanoic acid (b), tridecanoic acid (c), azelaic acid (d), decanedioic acid (e), undecanedioic acid (f), dodecanedioic acid (g) and tridecanedioic acid (h).