Supporting information

A sustainable synthetic route for biobased 6-hydroxyhexanoic acid, adipic acid and ε-caprolactone by integrating bio- and chemical catalysis

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Experimental section

Materials

5-Hydroxymethylfurfural (5-HMF, 99% purity), 1,6-hexanediol (1,6-HD), 6-hydroxyhexanoic acid (6-HHA), adipic acid (AA), ε-caprolactone (ε-CL), dimethylformamide, (D)-fructose (99% purity), molecular sieves (4Å), and Dowex DR-2030 hydrogen form (strong cation ion exchange resin) were procured from Sigma-Aldrich. DOWEX® DR-2030 is a macroporous styrene-divinylbenzene resin functionalized with sulfonic acid groups, >4.7 meq/g dry weight capacity, 16-40 mesh, 30 m^2/g surface area, and <3% moisture content, with upper limit for safe operation at 130 °C. All chemicals were used without further treatment.

Preparation of HMF

5-HMF was prepared according to our previous report using 100 mL of 30% w/w fructose in DMSO and 6 g ion exchange resin DR2030 at 110 °C, followed by purification by liquid-liquid extraction, evaporation and flash chromatography [7]. Purified 5-HMF (95% purity determined by ^{1}H-HMR) was obtained with an overall recovery yield of 72%.

One-pot cascade microbial oxidation of 1,6-HD to AA through 6-HHA

Lyophilized cells of *G. oxydans* DSM 50049 were inoculated into 50 mL *Gluconobacter* broth medium in a 250 mL flask, containing (per liter): 100 g glucose and 10 g yeast extract at pH 6.8. The flask was incubated in a shaker incubator (Ecotron, Infors HT, UK) at 30 °C and 200 rpm for 24 h. Then 20 % (v/v) glycerol stock of the bacterial culture was prepared by mixing 500 µL culture broth with 500 µL of 40 % glycerol solution, and stored at -20 °C for further use. For preparing the cells for transformation of 1,6-HD, 100 µL glycerol stock of *G.*

oxydans was inoculated into 50 mL medium in a 250 mL flask, containing per liter: 25 g glycerol and 10 g yeast extract with pH adjusted to 5, and incubated as described above. Thereafter, the culture broth was centrifuged at 4700 × g for 15 min (Sorvall LYNX 4000, Thermo Scientific, Germany), the cell pellet was separated and washed twice using 0.1 M sodium phosphate buffer pH 7 prior to use in the oxidation reactions.

The cell pellet collected from 2 mL cultivation medium, was re-suspended in 1 mL of 100 mM sodium phosphate buffer (pH 7) supplemented with a given concentration of 1,6-HD in 4 mL vials. All vials were incubated in a thermomixer (MKR 13, HLC Biotech, Germany) at 30 °C and 700 rpm without pH control. Twenty microliter samples were collected during the course of the reaction for analyzing substrate and product concentrations.

In order to purify AA obtained from 1,6-HD, the cell pellet of *G. oxydans* DSM 50049 (40 mg cell dry weight) was collected from 20 mL cultivation medium and suspended in 10 mL of 100 mM sodium phosphate buffer (pH 7) supplemented with 10 mg/mL 1,6-HD in a 50 mL tube. The biotransformation was performed in an incubator at 30 °C and 200 rpm for 40 hours. The resulting reaction mixture was centrifuged at 11 000 × g to remove the cells, and followed by simple evaporation of the supernatant to remove water. The dried residue was suspended in methanol (to remove the salt), and the methanol solution was dried by simple evaporation. AA was obtained with 95.5% isolated yield after washing the dry solid with cold water and vacuum drying, and was confirmed by H-NMR.

***Batch and continuous selective oxidation of 1,6-HD to 6-HHA by G. oxydans* DSM 50049**

The cell pellet of *G. oxydans* DSM 50049 was collected from 20 mL cultivation medium and suspended in 10 mL of 100 mM sodium phosphate buffer (pH 7) supplemented with 84.6 mM 1,6-HD in a 50 mL tube. The biotransformation was performed in an incubator at 30 °C and

200 rpm for 30 h under pH control (pH 6–7) using 0.5 N NaOH solution. The resulting reaction mixture was centrifuged at 11 000 × g to remove cells, followed by simple evaporation to remove water. The dried residue was treated with methanol leaving the salt residue, and the methanol solution was subjected to flash silica chromatography. 6-HHA was obtained with 96.5% isolated yield after concentration and vacuum drying, and confirmed by H-NMR.

Using the same procedure as above, the cell pellet of *G. oxydans* DSM 50049 was collected from 20 mL cultivation medium and suspended in 10 mL of 100 mM sodium phosphate buffer (pH 7) supplemented with 84.6 mM 1,6-HD in a 50 mL tube. The biotransformation was performed in an incubator at 30 °C and 200 rpm under pH control (pH 6–7) using 0.5 N NaOH solution. Twenty microliter samples were collected during the course of the reaction for analyzing substrate and product concentrations. After checking the concentration, 100 μL of 50 g/L 1,6-HD (50 mg) was added at 24, 44, 60, and 72 h, each in a fed-batch mode.

***Selective oxidation of 1,6-HD to 6-HHA by Mycobacterium sp.* MS16**

The selective oxidation of 1,6-HD to 6-HHA was also performed by *Mycobacterium sp*. MS16, which was reclassified from *Corynebacterium* sp. ATCC 21245 [38,39]. To prepare the stock culture [39], 1 mL of the microbial culture in nutrient broth was transferred to 50 mL of sterile production medium containing per liter: 10 g yeast extract, 5 g glycerol, and 2 g sodium acetate (pH 7) in a 250-mL Erlenmeyer flask. The flask was incubated in an orbital-shaking incubator (New Brunswick, Innova 4430, Edison, USA) at 31°C and 250 rpm for 48 h. The resulting culture was mixed with sterile glycerol (20% v/v final concentration) and was stored at –80 °C. Preparation of the preculture was performed by transferring 0.6 mL of the stock culture in glycerol to 50 mL of production medium in a 250-mL Erlenmeyer flask. The

culture was placed in an orbital-shaking incubator at 31°C and 250 rpm for 48 h, and was then used as inoculum for batch cultivations performed under similar conditions as the oxidation of 1,6-HD to AA by *G. oxydans*.

Catalytic cyclization of 6-HHA to ε-CL

Typically, 50 mg 6-HHA was dissolved in 1.5 mL DMF, and placed in 4 mL glass vials, followed by addition of the ion exchange resin DR-2030 and molecular sieves. The solution was heated and stirred at 130 and 140 °C, respectively. Effect of varying weight ratios of ion exchange resin (DR-2030) and molecular sieves at the same concentration of 6-HHA on conversion and product selectivity at 140 °C was evaluated. Samples (20 μL each) were collected at different time intervals and analyzed for concentrations of 6-HHA and products. For production of ε-CL, 50 mg 6-HHA obtained from oxidation of 1,6-HD by *G. oxydans* DSM 50049 in the above section was dissolved in 1.5 mL DMF, and placed in a 4 mL glass vial, followed by addition of 50 mg ion exchange resin DR-2030 and 1.5 g molecular sieves. The solution was heated and stirred at 140 °C for 6 h. The reaction mixture was concentrated by simple evaporation at 50 °C; ε-CL and the co-product ε-formyloxyhexanoic acid (ε-FOHA) were separated by silica column chromatography using ethyl acetate:n-hexane (5:2) as the mobile phase. An overall isolated yield of ε-CL from 6-HD was 67.2%, and the purified ε-CL was analyzed by ^{1}H-NMR.

Analytical procedures

Cell density was determined by measuring the optical density of cell broth at 620 nm using UV/Vis Spectrophotometer (Ultrospec 1000, Pharmacia Biotech, Sweden). The cell dry weight (CDW) was determined by collecting cells from 1 mL fermentation broth at 4700 ×g

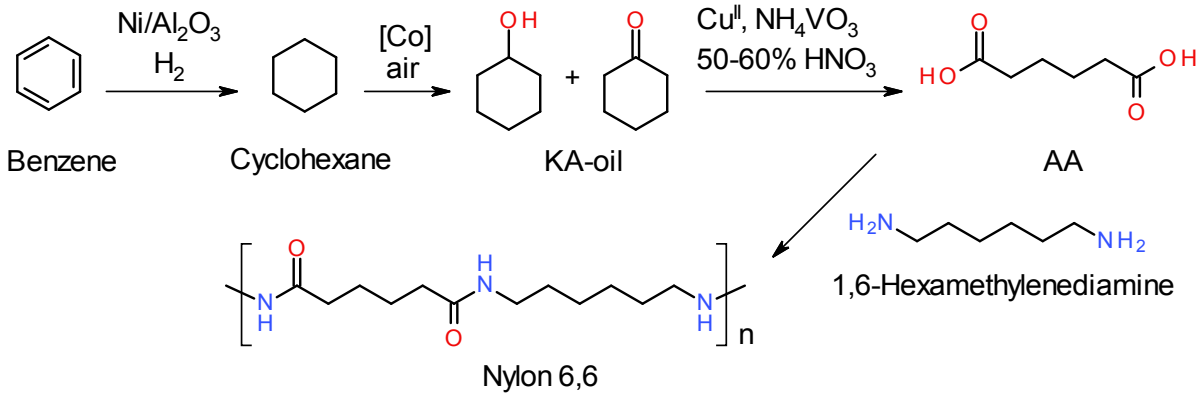
for 10 min in a dried pre-weighed 1.5 mL Eppendorf tube. The collected cell pellet was dried overnight at 105 °C. The increase in weight of the tube equals CDW per milliliter. The OD_{620nm} was correlated to CDW by the following equation:

$$CDW\ (g\ L^{-1}) = OD_{620} \times 0.4 \qquad (Eq.\ 1)$$

Quantitative analyses of reaction components were performed using gas chromatography (GC, Varian 430-GC, Varian, USA) equipped with FactorFour Capillary column, VF-1ms (Varian, 15M × 0.25 mm) and a flame ionization detector. The initial column oven temperature was increased from 50 to 250 °C at a rate of 20 °C/min. The samples, diluted with acetonitrile (0.1% DMSO as external standard), to a concentration of 0.1–0.5 mg/mL, were injected in split injection mode of 10 % at 275 °C. Conversion of the substrates and concentration of products formed were calculated from the standard curves on the gas chromatograms. The selectivity of products was calculated from the amount (mmol) of product formed and consumed substrate (Selectivity (%) = (product (mmol) / consumed substrate (mmol)) x 100). Isolated yields of the products were calculated according to the molar ratio of isolated products to used substrates for representative reactions.

Structures of the products were determined by ^{1}H and ^{13}C-NMR using 400 MHz NMR (Bruker, UltraShield Plus 400, Germany). Gas Chromatography-Mass Spectrometry (GC-MS, 431-GC and 210-MS, Varian, USA) run under the same conditions as the GC analysis described above.

Scheme S1. Commercial adipic acid production from petroleum-derived benzene, and its use in the production of Nylon 6,6. Oxidation of cyclohexane using Co catalyst (cobalt- (II) naphthenate) and air as an oxidant to KA oil, a mixture of cyclohexanone and cyclohexanol, is typically conducted at low conversions (3 to 8%) to maintain high selectivity (70−90%), necessitating extensive feed recycling and huge capital costs [19]. Further oxidation of KA oil to adipic acid occurs under harsh conditions using nitric acid, with co-production of undesired N_2O.



Scheme S2. Oxidation pathway of 1,6-hexanediol to adipic acid via different oxidative intermediates

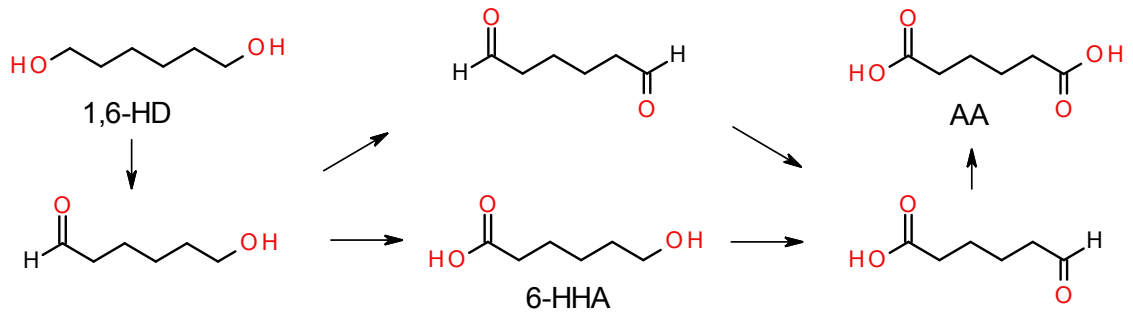


Figure S1. One-pot oxidation of 42.3, 84.6, 126.9 and 169.2 mM 1,6-hexanediol (1,6-HD) to adipic acid (AA) via 6-hydroxyhexanoic acid (6-HHA) at 30 °C by *Gluconobactor oxydans* DSM 50049. Conversion (%, ■) of 1,6-HD and concentration (mM, ▲) of AA at 36 h of reaction are shown.

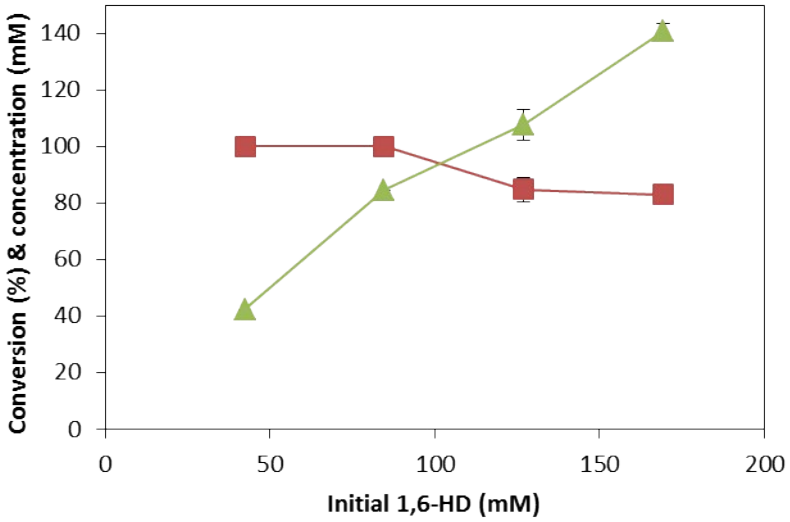


Figure S2. One-pot cyclization and esterification of 6-hydroxyhexanoic acid (6-HHA) by combination of two acidic heterogeneous catalysts. Effect of amount of molecular sieves on the selectivity of ε-caprolactone formation. Reaction conditions: 0.38 mmol 6-HHA in 1.5 mL DMF at 140 °C using various amounts of molecular sieves and 50 mg cation exchange resin DR-2030.

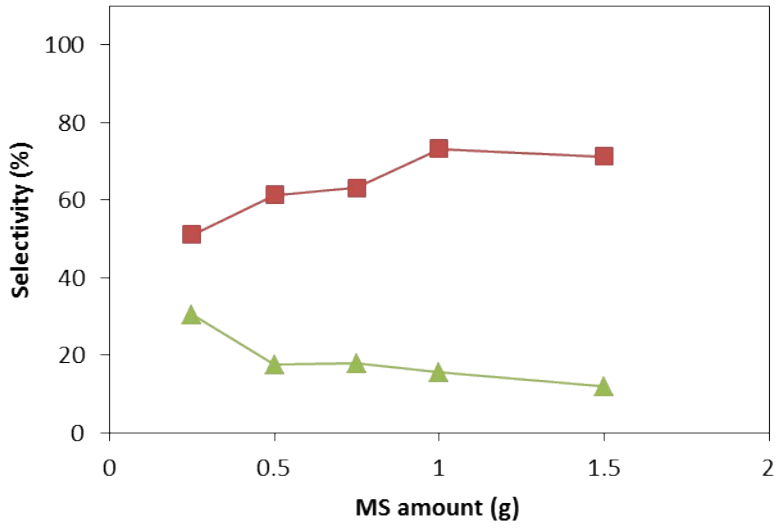


Figure S3. GC-MS chromatograms for representative microbial oxidation of 42.3 mM 1,6-hexanediol (1,6-HD) to adipic acid (AA) via 6-hydroxyhexanoic acid (6-HHA) at (A) 3 h, (B) 12 h and (C) 36 h, and (D) cyclization of 6-HHA to ε-caprolactone (ε-CL).

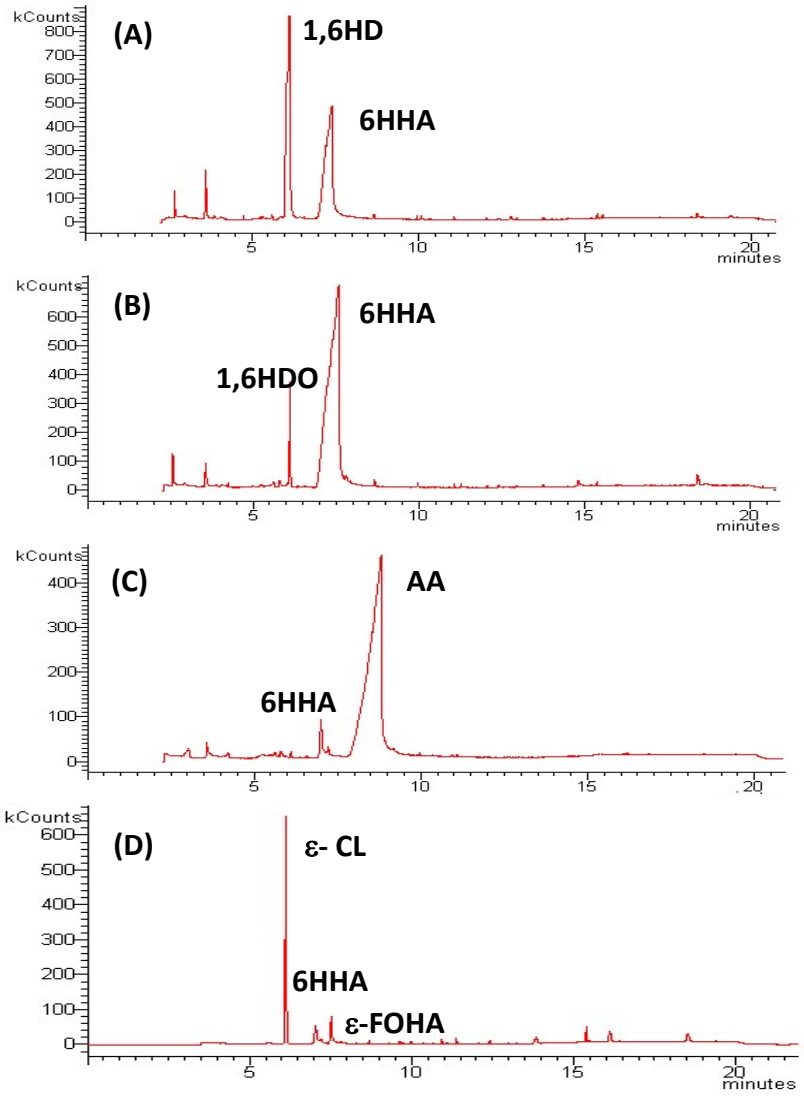


Figure S4. Molecular masses of 1,6-hexanediol (1,6-HD), 6-hydroxyhexanoic acid (6-HHA), adipic acid (AA), ε-caprolactone (ε-CL) and ε-(formyl)oxyhexanoic acid (ε-FOHA, -CHO) analyzed by GC-MS.

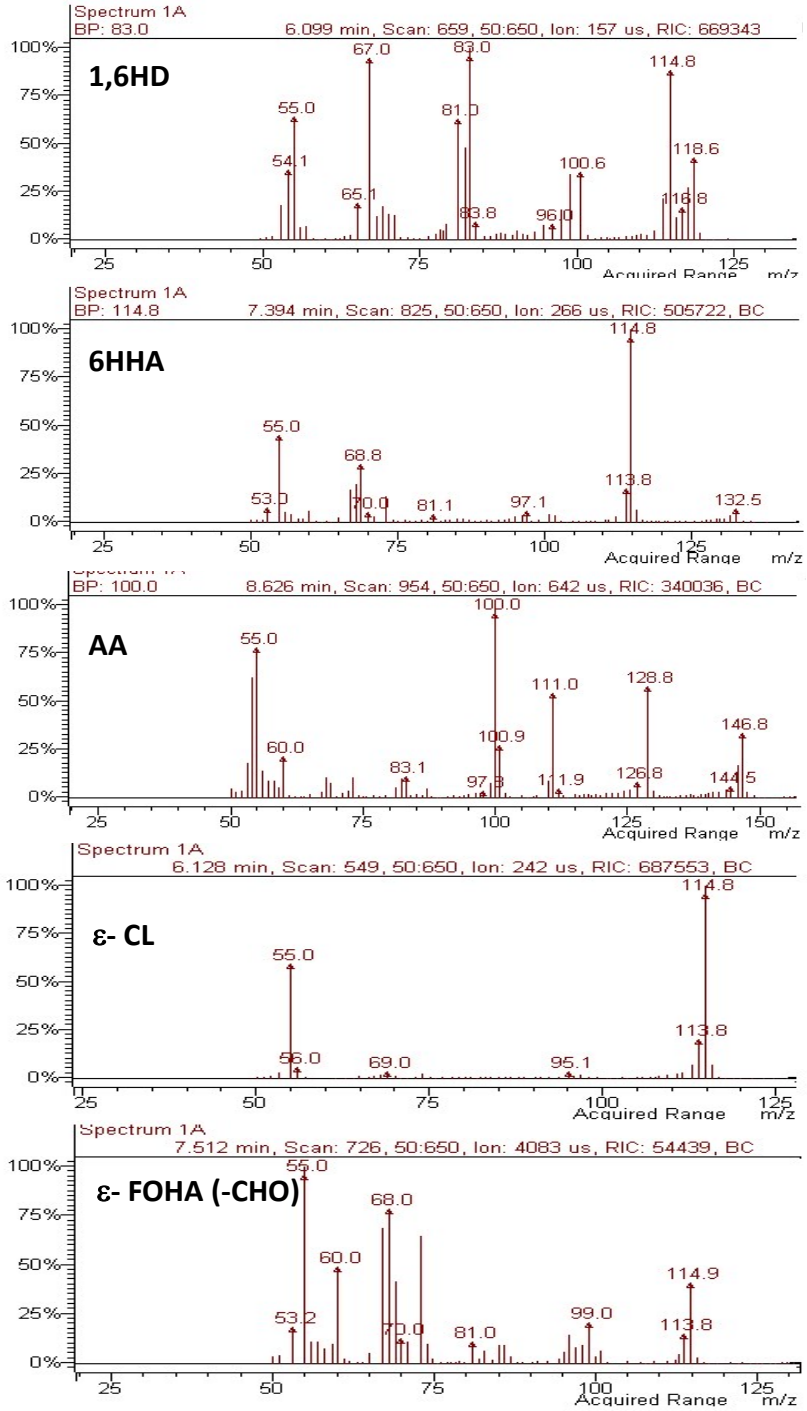


Figure S5. (A) ^{1}H-NMR, (2) ^{13}C-NMR, and (C) COSY-NMR of ε-(formyl)oxyhexanoic acid (ε-FOHA) by 400 MHz NMR ($CDCl_3$).