Supporting Information

Adding Value to Renewables: A One Pot Process Combining Microbial Cells and Hydrogen Transfer Catalysis to Utilise Waste Glycerol from Biodiesel Production

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Experimental.

Organism and culture conditions.

Clostridium butyricum was re-isolated from the freeze-dried culture of DSM10703 provided by Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, since the culture produced two colony morphologies on RCM agar. The larger, spreading colonies were selected for use, since they produced 1,3-propanediol more quickly than the small, compact colonies (data not shown). Cultures were grown at 30ºC in an anaerobic cabinet (DW Scientific) except where stated, and stock cultures were maintained on Reinforced Clostridial Medium (RCM) agar (Sigma). Experimental cultures were grown at 2.5 L scale in Y5 medium (modified from ref. [1]) at 35 °C, and contained (per l): pure or crude glycerol (20 g), KH_2PO_4 (0.5 g), K_2HPO_4 (0.5 g), $CoCl_2.6H_2O$ (0.01 g), NH_4Cl (1.65 g/L) and yeast extract (3 g). $MgSO_4.7H_2O$ (0.2 g) was autoclaved separately, and $FeSO_4.7H_2O$ (0.01 g) was filter sterilized anaerobically, and both were added to the sterile medium. The pH was controlled at pH 6.5 by automatic addition of 2M KOH. The medium was sparged with N_2 for at least 2 h before adding a 5% (v/v) inoculum. The crude glycerol (Lot Number A3996) from a biodiesel process was supplied by Lambson Ltd (Wetherby, Yorkshire, UK). The crude glycerol contained sulphated ash (6.72%), glycerol (91.5%), water (0.1%), and non-glycerol organic materials (1.72%).

Analysis of fermentation products.

Initial and final glycerol concentrations and concentrations of fermentation products (including 1,3-propanediol) were measured by HPLC (modified from ref. [2])using an Aminex HPX-87H Column (300 x 7.8 mm , Bio-Rad) with 5mM sulphuric acid as the mobile phase (0.5 ml/min) at 30°C with a refractive index detector.

Preparation of culture supernatants for reaction with catalysts.

The cultures of *C. butyricum* were harvested after 1,3-propanediol production had stopped, and were centrifuged at 13,000 x g and 4 ^{O}C to remove the cells. The resulting supernatant was then stored at 4 ^{o}C before use in the catalytic amination of 1,3-propanediol.

Chemical reagents and procedures.
All solvents used were dried using standard methods and stored over molecular sieves (4Å) under dinitrogen/argon. All reactions were performed under the protection of dinitrogen/argon. Anhydrous toluene and ionic liquid $N_{1,8,8,8}NTf_2$ used as solvents for the amination were purchased from the Aldrich. The compounds, aniline, 1,3-propanediol (99%), 1,1-bis(diphenylphosphino)ferrocene (97%), (dppf) 1,2-bis(diphenylphosphino)methane (98%) (dppm) and 1,3-bis(diphenylphosphino)propane (98%) (dppp), and complex $[(\eta^6\text{-cymene})RuCl(\mu\text{-}Cl)]_2$ were used as supplied (Aldrich). The complexes $[Cp^*IrCl(\mu\text{-}Cl)]_2$,[3] **a**[4] and **b**[5] were prepared as described in the literature. The precursor reagents for complexes preparation were used as received.

All the products were characterized by ^1H, ^{13}C NMR and MS, and comparison of their NMR spectra has been made with available literature data. ^1H and ^{13}C NMR spectra were recorded in ppm using Bruker DPX300 (300 MHz) or DRX500 (500 MHz) spectrometers, with reference to TMS internal standard (δ 0). LSIMS and ES mass spectra were recorded on a VG Autospec X series mass spectrometer.

Preparation of complexes a[4] and b[5]:
Complex a: A solution of 1,3,4,5-tetramethylimidazol-2-ylidene, freshly generated from 1,3,4,5-tetramethylimidazole-2(3H)-thione (0.204 g, 1.30 mmol) which was prepared by literature procedure[6] and potassium (0.130 g, 3.85 mmol) in THF (10 mL) was slowly added to a suspension of $[Cp^*IrCl(\mu\text{-}Cl)]_2$ (0.502 g, 0.65 mmol) in THF (15 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 20 min. The solvent was removed by evaporation and the residue was dissolved in CH_2Cl_2. This solution was filtered through a pad of activated carbon. Evaporation of the solvent gave orange-yellow powder which was recrystallized by a slow diffusion of *n*-pentane into dichloromethane to give orange crystals of **a** (0.234 g, 0.45 mmol, 36%). ^1H NMR ($CDCl_3$, δppm): 3.82 (s, 6H), 2.16 (s, 6H), 1.62 (s, 15H). ^{13}C NMR ($CDCl_3$, δppm): 153.98 (Ir-C), 125.92 (C=C), 88.30 (C_5Me_5), 35.83 (NMe), 9.61 (C_5Me_5), 9.18 (C=CMe).
Complex b: 1-Benzyl-3-methylimidazol-2-ylidene silver bromide (0.266 g, 0.37 mmol), freshly prepared from 1-benzyl-3-methylimidazolium bromide and Ag_2O, was added to $[Cp^*IrCl(\mu\text{-}Cl)]_2$ (0.297 g, 0.37 mmol) in CH_2Cl_2 (100 mL) and left for 15 h at room temperature. The solution was filtered through celite, which was washed with CH_2Cl_2 (3 x 10 mL). The filtrate and the washings were combined and the solvent was removed by rotary evaporation to yield a yellow powder which was recrystallized by slow diffusion of *n*-pentane into dichloromethane to give complex **b** as orange crystals (0.273 g, 0.48 mmol, 65% yield). ^1H NMR ($CDCl_3$, δppm): 7.34 (m, C_6H_5, 5H), 6.90 (d, J = 2.0 Hz, imidazolyidene-H, 1H), 6.69 (d, J = 2.0 Hz, imidazolyidene-H, 1H), 6.05 (d, J = 14.7 Hz, NCH, 1H), 5. 20 (d, J = 14.7 Hz, NCH, 1H), 4.01 (s, NMe, 3H), 1.64 (s, Cp*, 15H).

Amination of pure 1,3-propanediol (99%) with aniline.
The mixture of aniline (93.6 mg, 1.0 mmol), 1,3-propanediol (38.1 mg, 0.5 mmol), K_2CO_3 (6.8 mg, 0.05 mmol) and catalyst **b** (5.7 mg, 0.01 mmol, 1% of –OH group) in toluene (0.5 mL) in a sealed reaction tube was heated with stirring at 115 °C for 24 h. The reaction mixture was allowed to cool to room temperature, diluted by adding CH_2Cl_2 (1.0 mL), and filtered to remove the salt. The solvent in the filtrate was removed under reduced pressure. A small sample was taken for ^1H NMR analysis to

determine the reaction conversion and the proportion of the products. The reaction provided >99% conversion of 1,3-propanediol.

The reaction was scaled up to isolate the pure products and confirm their identities. The crude extract was purified by flash chromatography on silica gel using a mixture of ethyl acetate and hexane (1:3 to 1:0) as eluant. ^{1}H NMR spectra are given below.

Amination of 1,3-propanediol from fermentation of pure or crude glycerol:
Culture supernatants were used as the sole source of 1,3-propanediol. Supernatant 1, containing 127 mM 1,3-propanediol, was produced by fermentation of pure glycerol and supernatant 2, containing 134 mM 1,3-propanediol, was produced by fermentation of crude glycerol as described above. Both toluene and ionic liquid were used as the reaction solvents. In the case of toluene as a solvent, supernatant 1 (3.96 mL) or 2 (3.74 mL), containing 0.5 mmol of 1,3-propanediol, was injected dropwise into the solution of catalyst **b** (5.7 mg, 1% mol of –OH), aniline (93.6 mg, 1.0 mmol), K_2CO_3 (6.8 mg, 0.05 mmol) in toluene (0.5 mL) in a reaction tube under argon. The tube was sealed and heated to 115 °C with stirring and maintained at this temperature for a the time shown in Table 3 in the manuscript. After reaction, the reaction mixture was allowed to cool to room temperature, and extracted with hexane (6 x 5.0 mL) at 0 °C. The hexane extracts were combined and the solvent was removed under reduced pressure. The crude product mixture obtained was subjected to ^{1}H NMR for measuring the product distribution. The aqueous phase was collected and subjected to HPLC (see above) to detect the unreacted 1,3-propanediol and calculate the reaction conversion.

Using ionic liquid as the reaction solvent, catalyst **b** was dissolved in ionic liquid by heating to *ca* 80 °C under argon, and the mixture was allowed to cool to room temperature. The other reagents were injected into the mixture in sequence at room temperature and the mixture was heated to the temperatures and maintained for the times shown in Table 3 in the manuscript. After reaction, the reaction mixture was extracted with hexane (12 x 5 mL) to generate a three-phase system. The aqueous phase and the hexane extract were analysed as mentioned above. ^{1}H NMR of the ionic liquid phase showed neither residual substrate nor products.
Product **1**: ^{1}H NMR ($CDCl_3$, δppm): 7.19 (apparent t, J = 7.3 Hz, 2H), 6.72 (apparent t, J = 7.3 Hz, 1H), 6.65 (apparent d, J = 7.3 Hz, 2H), 3.82 (t, J = 6.5 Hz, 2H), 3.65 (broad s, 1H), 3.28 (t, J = 6.5 Hz, 2H), 1.89 (apparent quintet, J = 6.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, δppm): 125.73, 114.12, 109.58, 58.17, 38.43, 28.42.
MS: CI, m/z 152 $[M+H]^+$ (100), 134 $[M-OH]^+$ (73); HRLSMS, calc 152.1075, found 152.1074 $[M+H]^+$ for $C_9H_{14}NO$. For published spectra see ref. [7].
Product **2**: ^{1}H NMR ($CDCl_3$, δppm): 7.18 (apparent t, J = 7.5 Hz, 4H), 6.71 (tt, J = 0.9 Hz, 7.5 Hz, 2H), 6.62 (apparent d, J = 7.5 Hz, 4H), 3.7 (broad s, 2H), 3.26 (t, J = 6.5 Hz, 4H), 1.94 (pentet, J = 6.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, δppm): 148.22, 129.31, 117.54, 112.90, 42.08, 29.33.
MS: CI, m/z 227 $[M+H]^+$ (100), 143 $[M-OH]^+$ (73); HRLSMS, calc 227.1548, found 227.1542 $[M+H]^+$ for $C_{15}H_{19}N_2$. For published spectra see ref. [8].
Product **3**: ^{1}H NMR ($CDCl_3$, δppm): 7.17 (apparent t, J = 8.0 Hz, 2H), 6.68 (apparent t, J = 7.2 Hz, 1H), 6.60 (apparent dd, J = 1.10, 8.0 Hz, 2H), 3.6 (broad s, 1H), 3.08 (t, J = 7.3 Hz, 2H), 1.64 (apparent sextet, J = 7.3 Hz, 2H), 0.99 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, δppm): 148.920, 129.619, 117.478, 113.095, 46.212, 23.141, 12.046;
ES-MS: 136.121 $[M+H]^+$ (100), 118.065 $[M-NH_3]$; HRLSMS: 136.1121 found, 136.1126 Cal, $[M+H]^+$. For published spectra see ref. [9].

References
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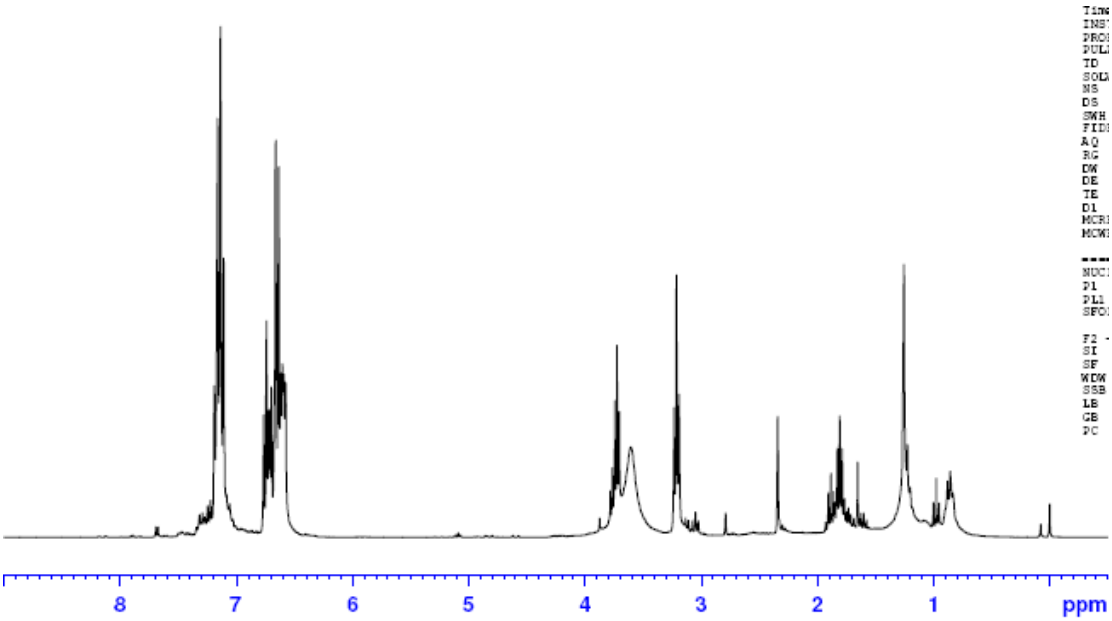
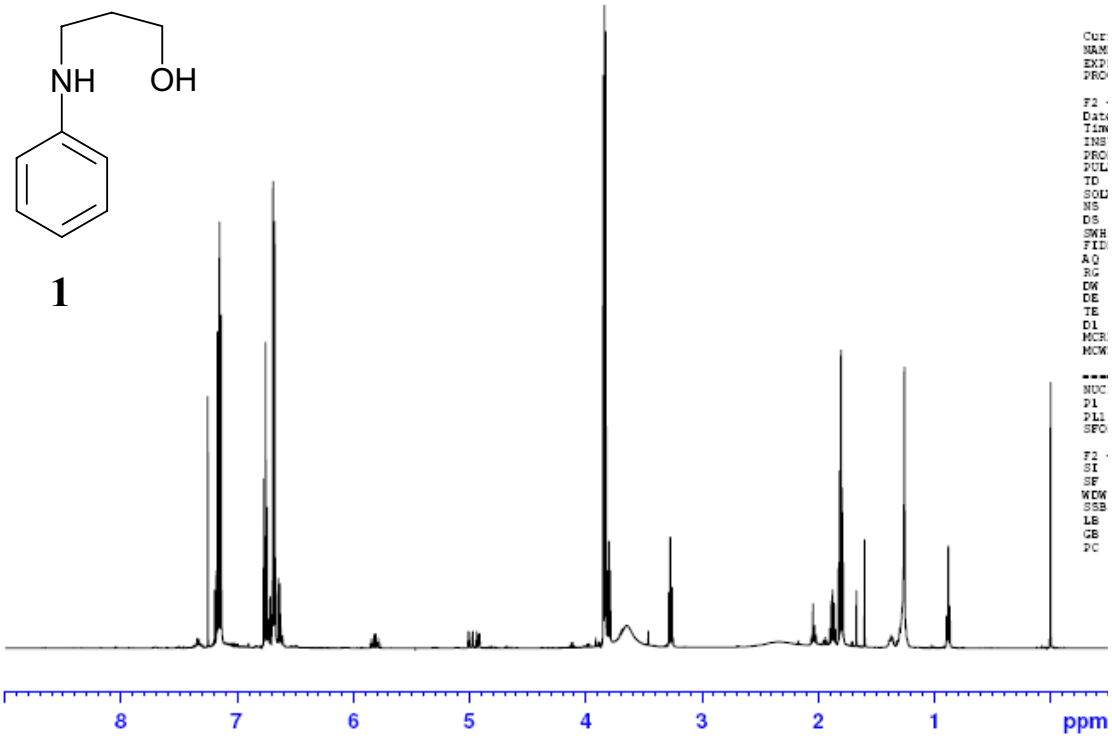
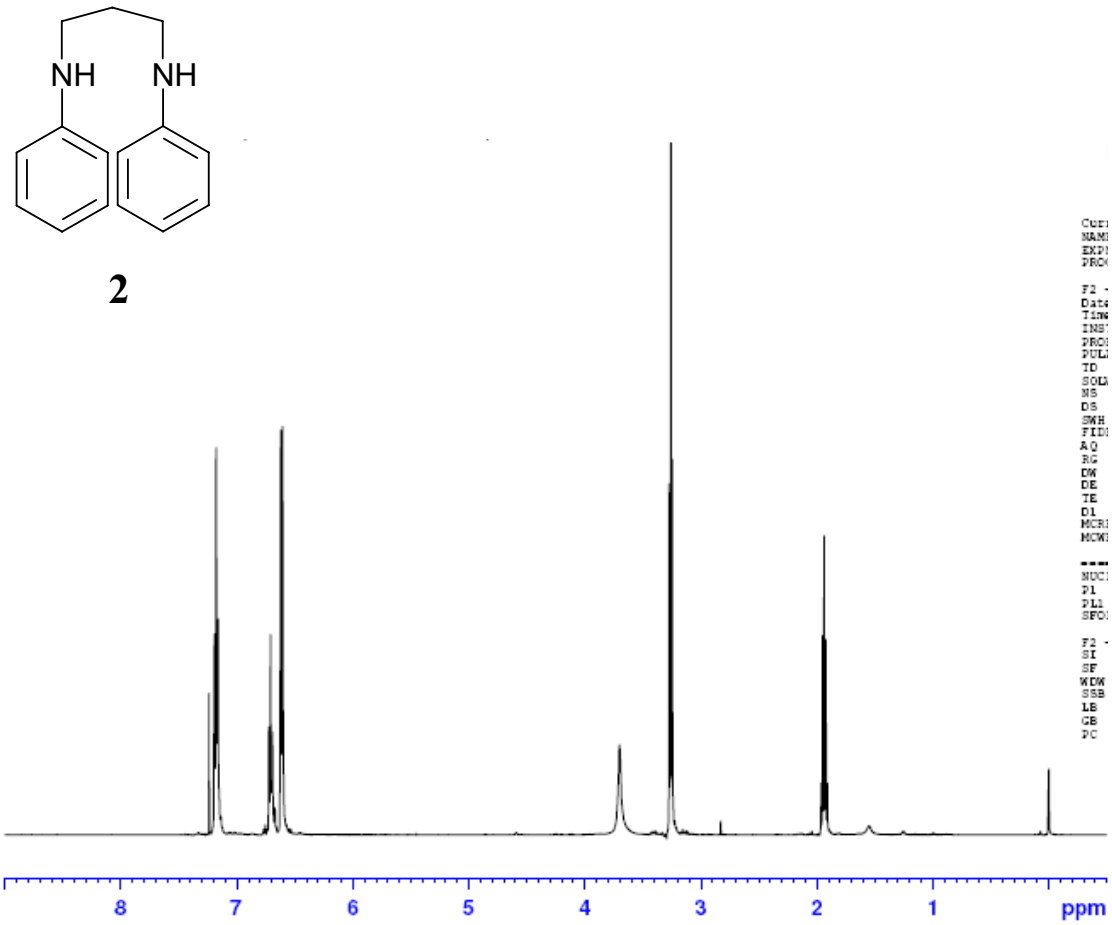
^{1}H NMR spectra of a reaction mixture and products 1, 2 and 3.



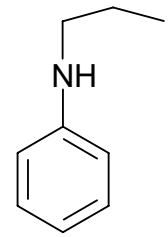
^{1}H NMR of a crude product mixture.



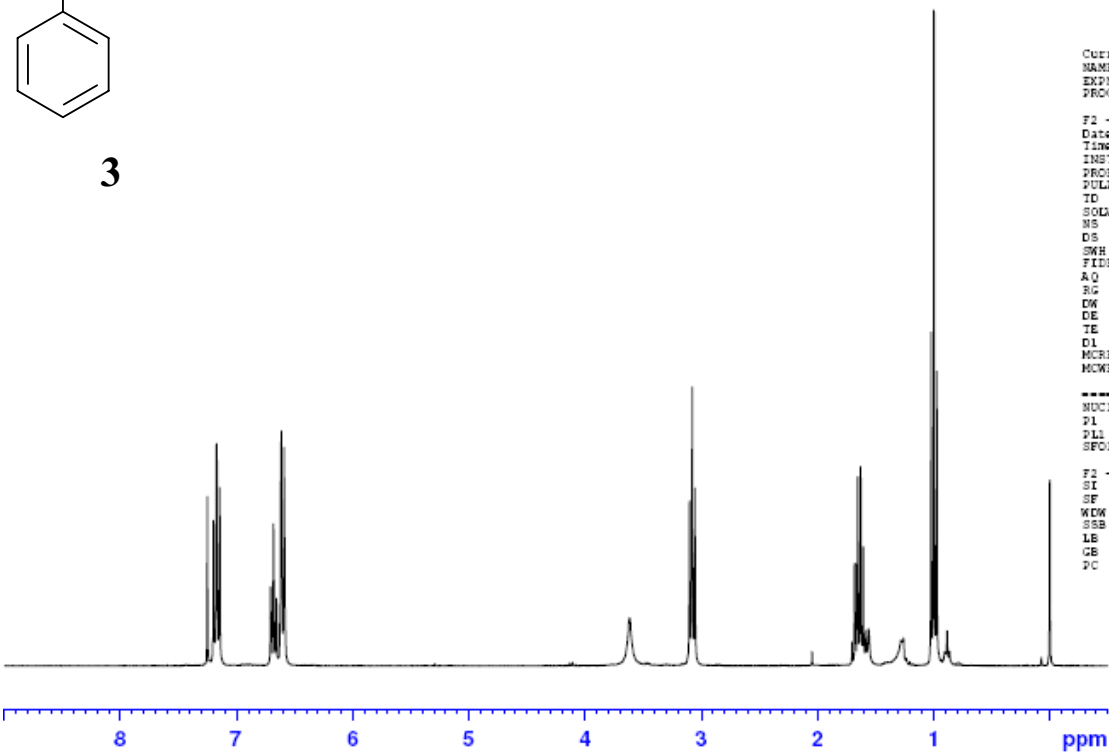
^{1}H NMR of **1** isolated from the amination of 1,3-propanediol.



^{1}H NMR of **2** isolated from the amination of 1,3-propanediol.



3



^{1}H NMR of **3** isolated from the amination of 1,3-propanediol.