Electronic Supplementary Information

Assessing the stereoselectivity of *Serratia marcescens* CECT 977 2,3-butanediol dehydrogenase

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1. Chemicals and enzymes

Chemicals were purchased from Sigma-Aldrich (Schnelldorf, Germany), Acros Organics (Geel, Belgium) and used without further purification (*meso*-2,3-butanediol, (2*S*,3*S*)-2,3-butanediol, (2*R*,3*R*)-2,3-butanediol, *rac*-acetoin, 2,3-butanedione (diacetyl), (±)-1,3-butanediol, (±)-2,4-butanediol, 2,3-pentanedione, 3,4-hexanedione, 1,2-cyclohexanedione, (±)-*cis/trans*-1,2-cyclohexanediol, (±)-*trans*-1,2-cyclohexanediol, (1*S*,2*S*)-*trans*-1,2-cyclohexanediol, (1*R*,2*R*)-*trans*-1,2-cyclohexanediol, 1-phenyl-1,2-propanedione, benzil, *rac*-benzoin, (*R*)-benzoin). Media components were sourced from BD (Becton Dickinson B.V., Breda, The Netherlands).

(*R*)-2-hydroxy-1-phenylpropan-1-one ((*R*)-HPP) and (1*R*,2*R*)-1-phenylpropane-1,2-diol ((1*R*,2*R*)-PPD) were produced following the procedure reported by Jakoblinnert and Rother (2014),[1] whereas (*S*)-2-hydroxy-1-phenylpropan-1-one ((*S*)-HPP) was obtained according to Kihumbu *et al.*, (2002).[2] (*R*)-1-hydroxy-1-phenylpropan-2-one ((*R*-PAC)) was obtained according to Seth *et al.*, (2012).[3] (1*S*,2*S*)-1-phenylpropane-1,2-diol ((1*S*,2*S*)-PPD)[4] and (*S*)-1-hydroxy-1-phenylpropan-2-one ((*S*)-PAC).[5]

NAD(P)H oxidase (PRO-NOX(001), 0.1 U mg^{-1}) and formate dehydrogenase (PRO-FDH(001), 0.4 U mg^{-1}) were acquired from Prozomix as crude extract formulations. Glucose dehydrogenase and ADH-380 were acquired from Evocatal (Monheim am Rhein, Germany).

2. Analytical methods

^{1}H and ^{13}C NMR spectra were recorded with an Agilent-400 MR DD2 instrument (400 MHz and 100 MHz, respectively). Chemical shifts (δ) are expressed in ppm, and coupling constants (J) are expressed in Hz. Optical rotations were determined at 25 °C with a Perkin-Elmer 241 polarimeter (sodium D line) and [α] values are in given in units 10^{-1} deg cm^2 g^{-1} and were in good accordance with reported data. Column chromatography was carried out with silica gel (0.060-0.200 mm, pore diameter ca. 6 nm) and Reveleris Silica Column (4g, Grace). Thin-layer chromatography (TLC) was performed on 0.20 mm silica gel 60-F plates. Compounds were visualized by treatment with a permanganate solution and heating.

Gas chromatography

α-Hydroxy ketone and diol formation and optical purity were monitored using chiral phase GC analysis. 1 µL of the samples (split 1/100) was analyzed using a chiral CP-Chirasil-DEX

CB column (Varian, Germany; 25 m × 0.25 mm × 0.25 µm). The operating conditions were as follows: helium was the carrier gas; the temperature of the injector and detector (flame ionization detector (FID)) were set at 250 °C and 275 °C, respectively. HPP and PAC stereoisomers were also analysed by chiral GC employing a Lipodex E column, (Macherey-Nagel, Germany, 50 m × 0.25 mm × 0.25 µm). The operating conditions were as follows: helium as carrier gas; the temperature of the injector and detector (FID) were set at 200 °C and 225 °C, respectively. GC programs and retentions times are detailed in Supplementary Table 1.

Supplementary Table 1: GC programs and retention times.

Column	**Program**[a]	**Compound**	**Retention time (min)**
A	60/7.5/10/85/1.5/10/100/1/10/ 125/1.5/25/225/1	diacetyl	3.0
		R-acetoin	9.7
		S-acetoin	10.6
		(*S*,*S*)-2,3-butanediol	16.5
		(*R*,*R*)-2,3-butanediol	16.8
		meso-2,3-butanediol	17.2
A	70/4/15/120/3/15/130/2/20/225/1	2,3-pentanedione	4.6
		(*R*)-2-hydroxypentan-3-one	8.2
		(*S*)-2-hydroxypentan-2-one	8.4
		(*S*,*S*)-2,3-pentanediol	11.7
A	70/2/15/130/5/15/225/1	2,3-hexanedione	4.8
		(*S*)-3-hydroxyhexan-2-one	8.1
A	70/4/15/130/5/15/225/2	3,4-hexanedione	6.0
		(*S*)-4-hydroxyhexan-3-one	9.6
		(*S*,*S*)-3,4-hexanediol	12.9
A	70/4/15/110/3/15/130/2/15/ 160/1/20/225/1	2,3-heptanedione	7.4
		(R)-2-hydroxyheptan-3-one	12.0
		(S)-2-hydroxyheptan-3-one	12.2
		3-hydroxyheptan-2-one	12.7
		(*S*,*R*)-2,3-heptanediol	15.9
A	140/30/25/225/1.5	1-phenyl-1,2-propanedione	4.8
		(*R*)-2-hydroxy-1-phenylpropan-1-one	8.8
		(*S*)-2-hydroxy-1-phenylpropan-1-one	9.3
		(*S*)-1-hydroxy-1-phenylpropan-2-one	10.1
		(*R*)-1-hydroxy-1-phenylpropan-2-one	9.3
		(1*S*,2*S*)-1-phenylpropane-1,2-diol	25.7
		(1*R*,2*R*)-1-phenylpropane-1,2-diol	27.4
		(1*S*,2*R*)-1-phenylpropane-1,2-diol	28.9
		(1*R*,2*S*)-1-phenylpropane-1,2-diol	29.7
B	90/3/8/140/8/20/220/1	(*R*)-2-hydroxy-1-phenylpropan-1-one	16.8
		(*S*)-2-hydroxy-1-phenylpropan-1-one	16.0
		(*S*)-1-hydroxy-1-phenylpropan-2-one	16.7
		(*R*)-1-hydroxy-1-phenylpropan-2-one	17.3

A	110/6/10/150/1/10/180/5/25/225/1	1,2-cyclohexanedione	7.8
		(*S*,*S*)-*trans*-1,2-cyclohexanediol	12.5
		cis-1,2-cyclohexanediol	12.7
		(*R*,*R*)-*trans*-1,2-cyclohexanediol	12.8

[a] Program: initial temperature (ºC) / hold time (min) / slope (ºC/min) / temperature (ºC) / hold time (min) / slope (ºC/min) / temperature (ºC) / hold time (min) / slope (ºC/min) / temperature (ºC) / hold time / slope (ºC/min) / temperature (ºC) / hold time. Column A: CP-Chirasil-DEX CB, column B: Lipodex E.

3. Synthesis of standards using Evoxx ADH-380 as biocatalyst

(a) Reduction of 2,3-pentanedione, **1a**.

After 24 h of incubation, (2*S*,3*S*)-2,3-pentanediol (**4a**) was chromatographed on a silica gel column using ethyl acetate as eluent with an overall yield of 12%. **^{1}H NMR** (400 MHz, Chloroform-*d*): δ 3.68-3.51 (m, 1H), 3.25 (m, 1H), 2.44 (br s, 2H), 1.61-1.50 (m, 1H), 1.45-1.34 (m, 1H), 1.18 (d, *J*=6.4 Hz, 3H), 0.98 (t, *J*=7.4 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 77.50, 70.51, 26.15, 19.45, 9.86.[6] $[\alpha]_D^{25} =$ -4.33 (*c* 0.94, $CHCl_3$). lit.[6] $[\alpha]_D^{22} =+$ 4.5 (*c* 1.0, $CHCl_3$) for (*R*,*R*)-2,3-pentanediol.

(b) Reduction of 3,4-hexanedione, **1c**.

After 48 h incubation, (3*S*,4*S*)-3,4-hexanediol (**4c**) was obtained in 25% isolated yield (eluent ethyl acetate). **^{1}H NMR** (400 MHz, Chloroform-*d*): δ 3.38-3.29 (m, 2H), 2.38 (s, 2H), 1.67-1.35 (m, 4H), 0.97 (t, *J*=7.5 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 75.46, 26.42, 9.96.[7] $[\alpha]_D^{25} =$ -15.8 (*c* 0.9, $CHCl_3$). lit.[7] $[\alpha]_D^{25} =$-16.5 (*c* 0.03, $CHCl_3$).

(c) Reduction of 2,3-heptanedione, **1d**.

Following a similar procedure, (*S*)-2-hydroxyheptan-3-one (**2d**) was obtained in 21 % yield after 48 h incubation. The acyloin was isolated by chromatography on a silica gel column (eluent petroleum ether: ethyl acetate 7:3). **^{1}H NMR** (400 MHz, Chloroform-d): δ 4.27-4.19 (m, 1H), 3.55 (d, *J*=4.7 Hz, 1H), 2.55-2.37 (m, 2H), 1.64-1.57 (m, 2H), 1.38 (d, *J*=7.1, 3H), 1.37-1.30 (m, 2H), 0.92 (t, *J*=7.3 Hz, 3H). **^{13}C NMR** (101 MHz, CDCl3): δ 212.64, 72.52, 37.21, 25.65, 22.32, 19.82, 13.75.[8] $[\alpha]_D^{25} =$ +50.85 (*c* 0.52, $CHCl_3$). lit.[9] $[\alpha]_D^{25} =+59.2°$, 88.7% *de* (*c* 0.9, $CHCl_3$).

(2*S*,3*R*)-2,3-heptanediol (**4d**) was eluted with ethyl acetate with an isolated yield of 7.3 %. **^{1}H NMR** (400 MHz, Chloroform-*d*): δ 3.60 (p, *J*=6.3 Hz, 1H), 3.38-3.29 (m, 1H), 2.24 (br s, 2H), 1.57-1.22 (m, 6H), 1.19 (d, *J*=6.3 Hz, 3H), 0.92 (t, *J*=7.1, 3H).[10] **^{13}C NMR** (101 MHz,

$CDCl_3$): δ 75.93, 70.61, 32.78, 27.44, 22.43, 19.23, 13.72. $[\alpha]_D^{25} =$ -18.2 (*c* 0.4, $CHCl_3$). lit.[11] $[\alpha]_D^{25}$ = -16.7 (*c* 0.035, $CHCl_3$).

4. Synthesis of standards using BudC as biocatalyst

(a) Reduction of 2,3-heptanedione, **1d**.

After 48 h reaction, acyloins (**2-3d**) were isolated using flash column chromatography (Reveleris X^2, Grace, The Netherlands) using Reveleris Silica column (4g) and mixture of petroleum ether: ethyl acetate 98:2 (v/v) as eluent with an overall yield of 20 %. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 212.64, 72.44, 37.04, 25.48, 22.17, 19.64, 13.62 corresponding to 2-hydroxy-3-heptanone (**2d**)[8] and δ 210.04, 76.70, 33.07, 26.72, 25.01, 22.36, 13.72 corresponding to 3-hydroxy-2-heptanone (**3d**).[12]

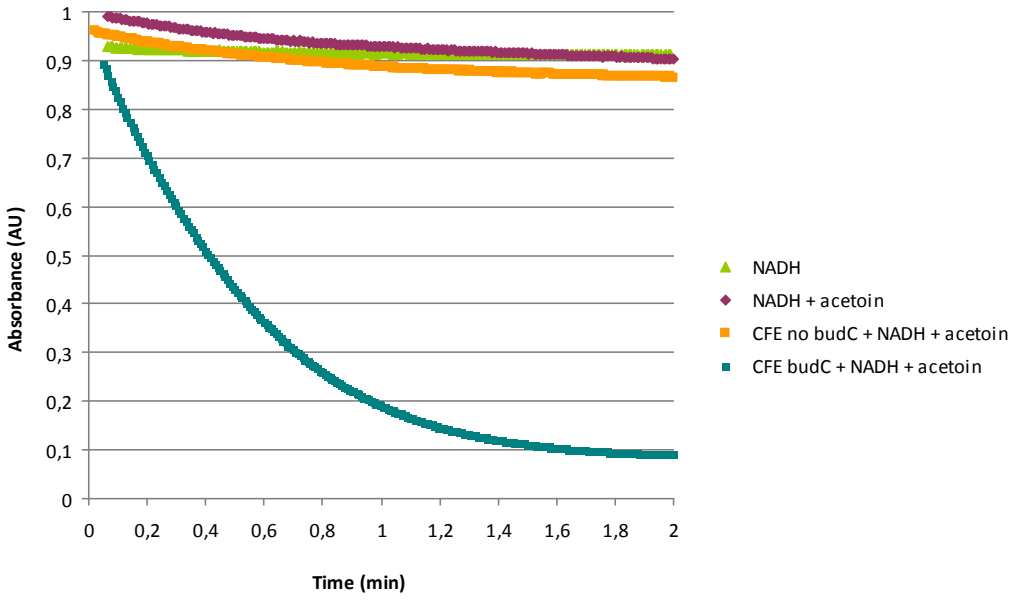
(b) Reduction of 3,4-hexanedione, **1c**.

After 48 h incubation, (*S*)-4-hydroxyhexan-3-one (**2c**) was isolated in 15% yield after purification on a silica gel column (eluent petroleum ether: ethyl acetate 9:1 (v/v)). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 4.20 – 4.12 (m, 1H), 2.58 – 2.34 (m, 2H), 1.88 (dqd, *J* = 14.1, 7.5, 4.0 Hz, 1H), 1.67 – 1.51 (m, 1H), 1.10 (t, *J* = 7.3 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 212.76, 76.96, 31.05, 26.81, 8.80, 7.55.[13] $[\alpha]_D^{25}$ =+ 98.2 (*c* 0.39, $CHCl_3$). lit.[14] $[\alpha]_D^{22}$ =-97.5 (*c* 1.0, $CHCl_3$) for (*R*)-4-hydroxyhexan-3-one.

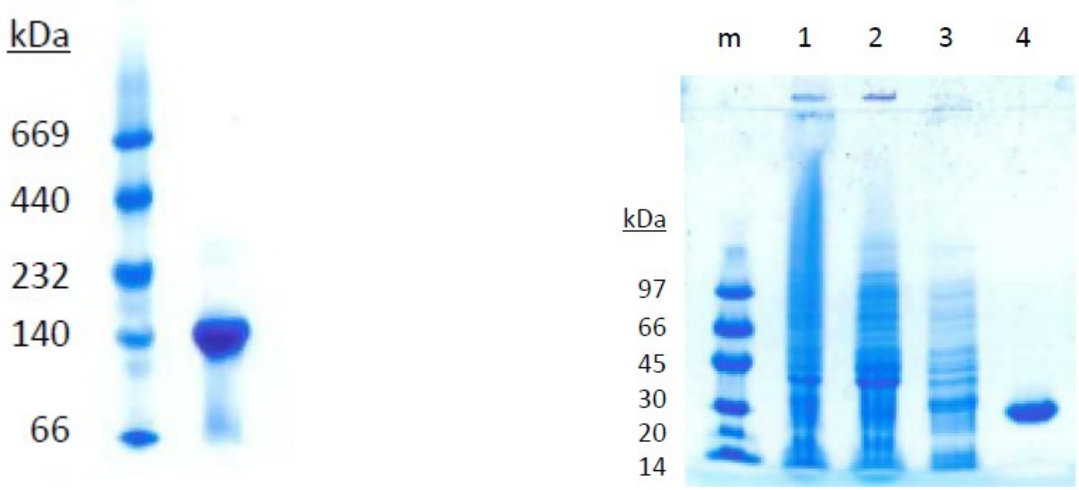
(c) Reduction of 2,3-hexanedione (**1b)**,

After 24 h reaction, (*S*)-3-hydroxyhexan-2-one (**3b**) was produced in 45% yield. Isolation was carried out using flash column chromatography (Reveleris X^2, Grace, The Netherlands) using Reveleris Silica column (4g) and petroleum ether: ethyl acetate mixture of 8:2 (v/v). **^{1}H NMR** (400 MHz, Chloroform-*d*): δ 4.12 (dd, *J* = 7.3, 3.7 Hz, 1H), 2.14 (s, 3H), 1.83–1.65 (m, 1H), 1.54 – 1.25 (m, 3H), 0.89 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): 210.06, 76.63, 35.55, 25.11, 18.04, 13.80.[15] $[\alpha]_D^{25}$ =+101 (*c* 0.6, $CHCl_3$). lit.[15] $[\alpha]_D^{25}$=-110.0 (*c* 0.8, $CHCl_3$) for (*R*)-3-hydroxyhexan-2-one.

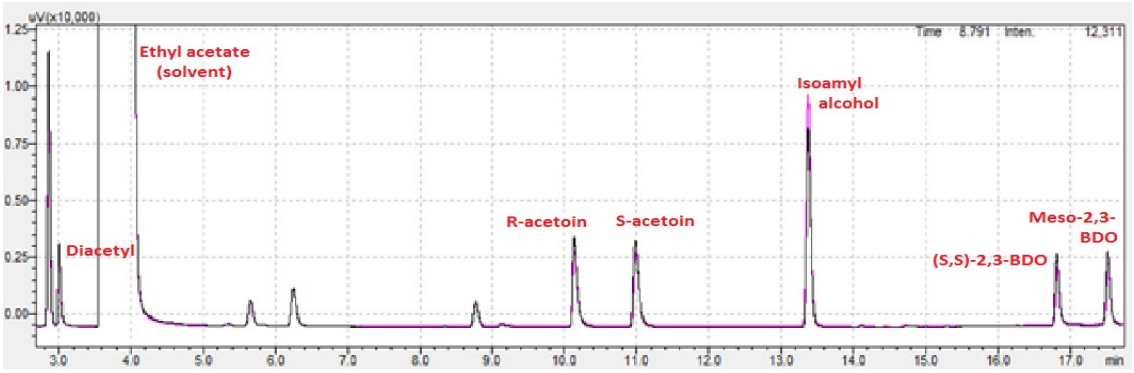
5. Supplementary Figures



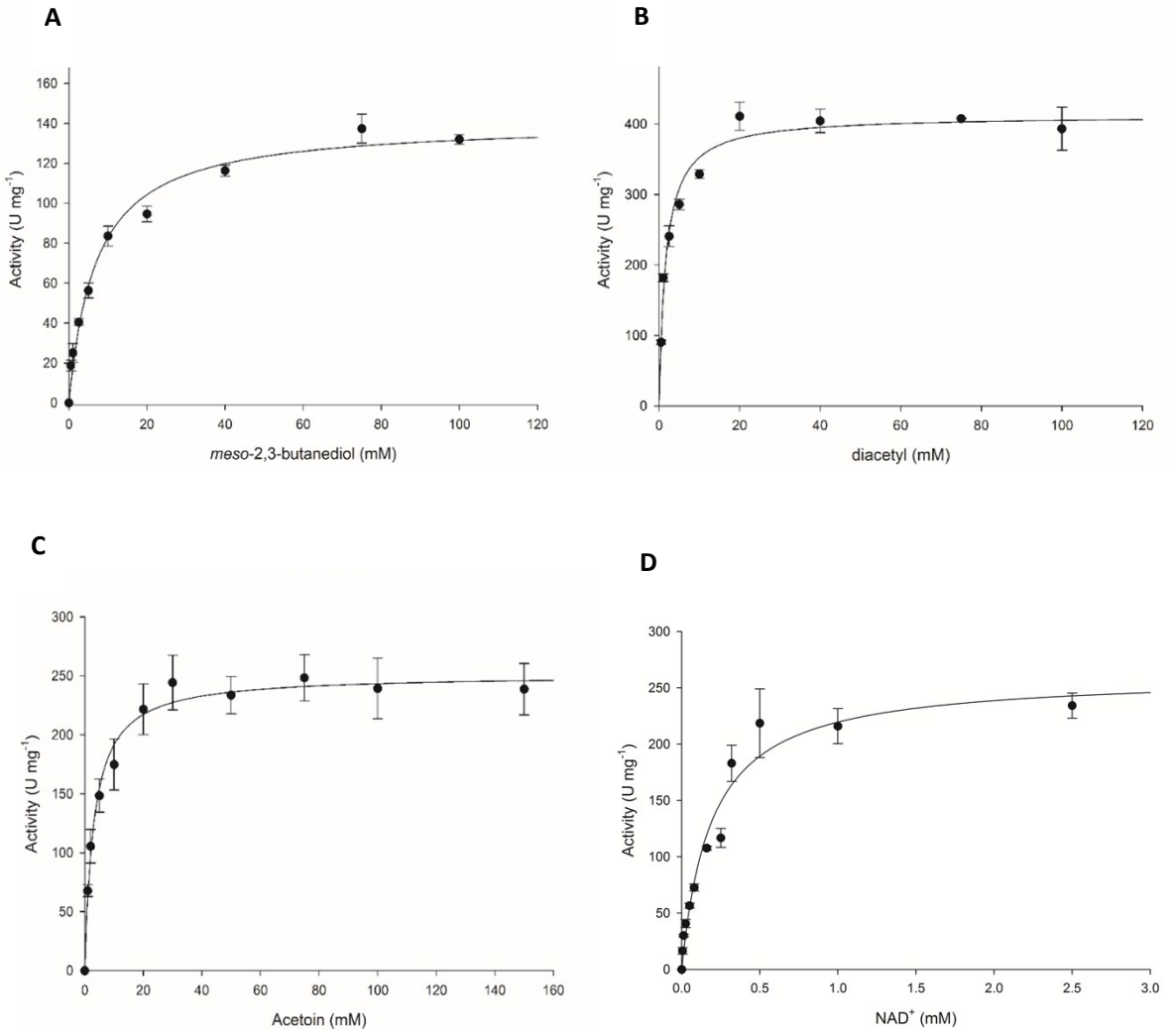
Supplementary Figure 1: BudC activity of *E. coli* BL21 star pET-28a *budC* cell-free extracts for *rac*-acetoin: 50 mM *rac*-acetoin, NADH 0.16 mM, enzyme solution (1 µl, 0.52 mg ml^{-1}) in potassium phosphate buffer (50 mM, pH 7.0). Cell-free extracts of *E. coli* BL21 star pET-28a (empty vector) were used as a negative control. 0.15 U mg^{-1} of dehydrogenase activity was determined in the latter protein solution.



Supplementary Figure 2: Coomassie Blue stained native (left) and SDS-PAGE (right) of BudC after recombinant gene expression in *E. coli* and subsequent protein purification, respectively. Native gel: lane m: protein marker, lane 1: purified BudC. SDS-PAGE gel: lane m: protein marker, lane 1: total protein, lane 2: soluble fraction, lane 3: insoluble fraction, lane 4: purified BudC.

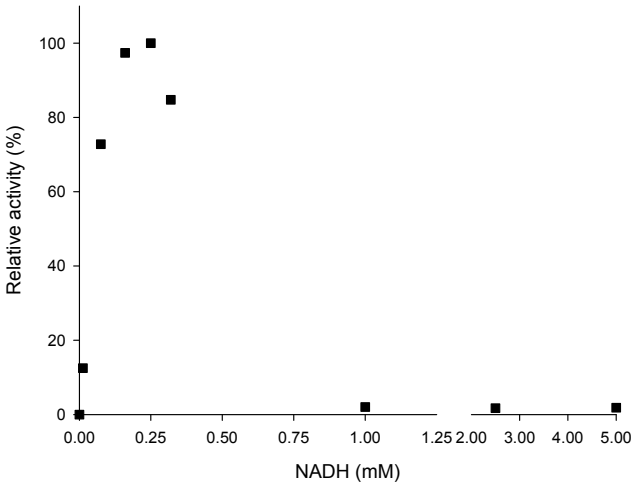


Supplementary Figure 3: Gas chromatogram of reaction standards at 5 mM.

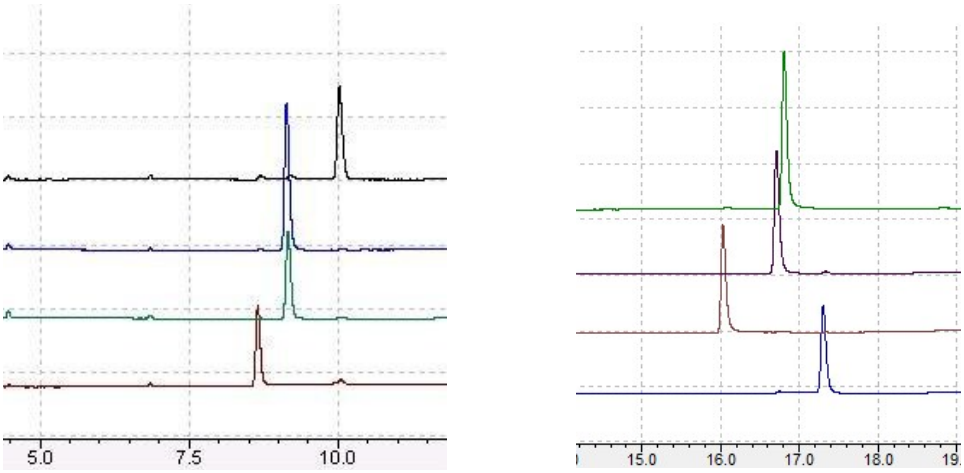


Supplementary Figure 4: BudC initial rates *vs* substrate/nicotinamide cofactor concentration A) *meso*-2,3-butanediol, B) diacetyl, C) acetoin, D) NAD^+. Michaelis-Menten curves of

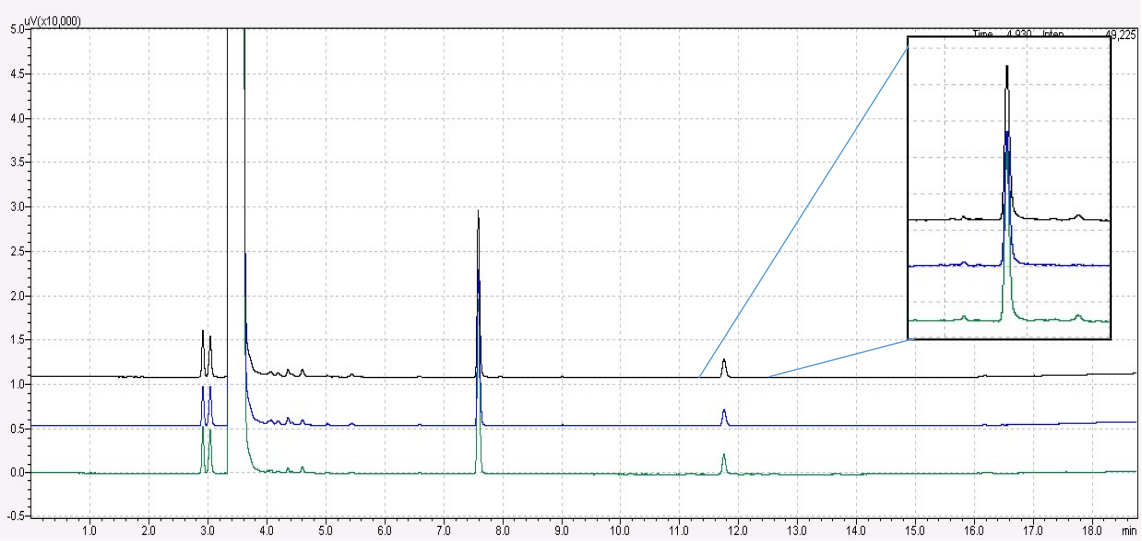
substrates converted by BudC at pH 7.0. Reaction conditions are described in the *Experimental Section*. Error bars represent the standard deviation of three independent experiments.



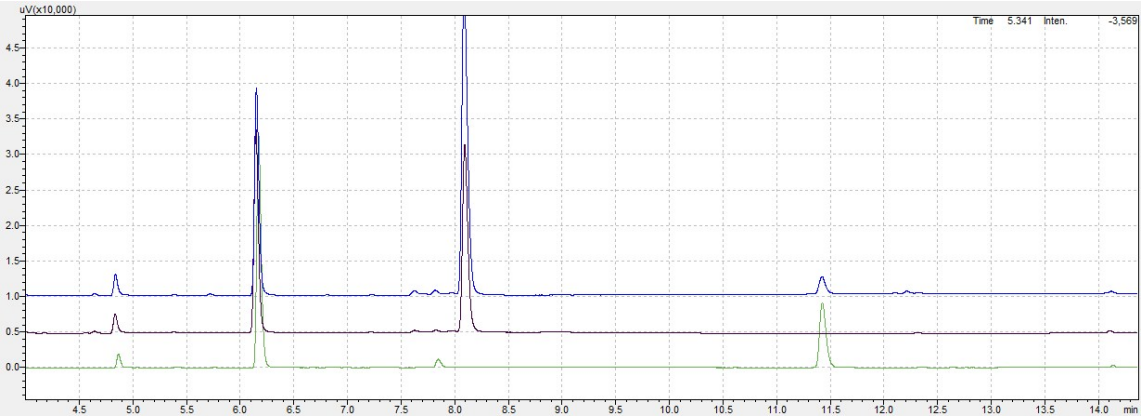
Supplementary Figure 5: BudC relative activity *vs* NADH concentration.



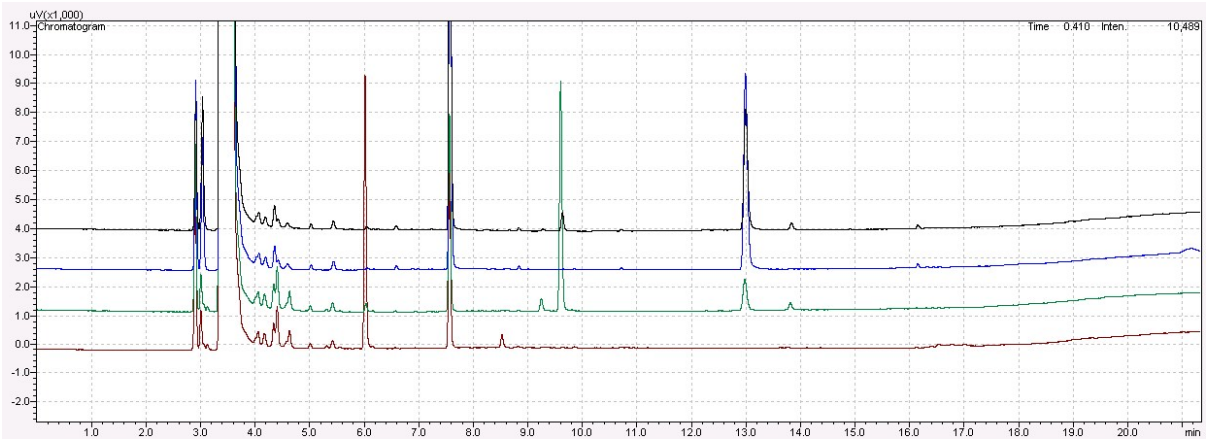
Supplementary Figure 6: GC traces *R*-, *S*-HPP and *S*-, *R*-PAC standards with Column A (left figure) and Column B (right figure); *R*-HPP, *S*-HPP, *R*-PAC, *S*-PAC.



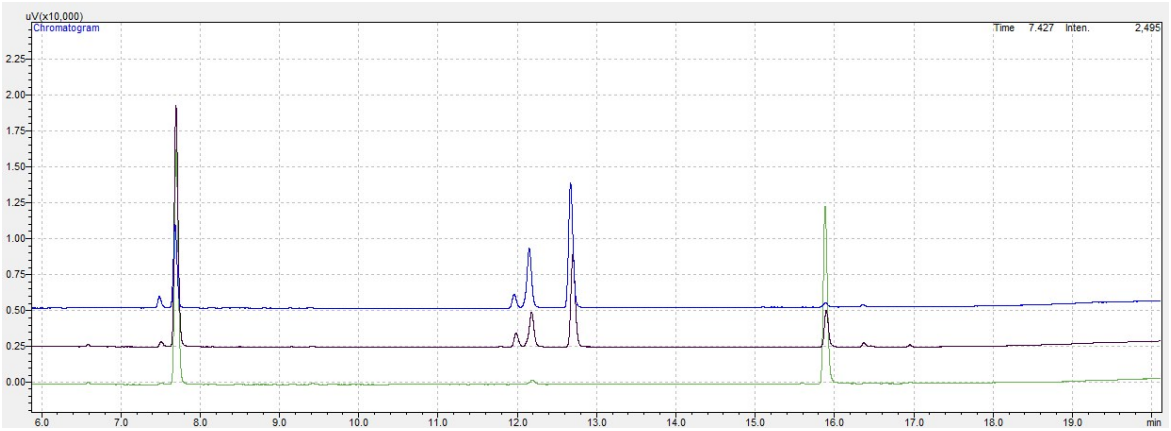
Supplementary Figure 7: GC chromatograms of 2,3-pentanedione (**1a**) standard reduction reactions catalysed by BudC (black and green traces) and ADH-380 (blue trace). Retention times: internal standard: 7.5 min; (2*S*,3*S*)-2,3-pentanediol (**4a**): 11.7 min.



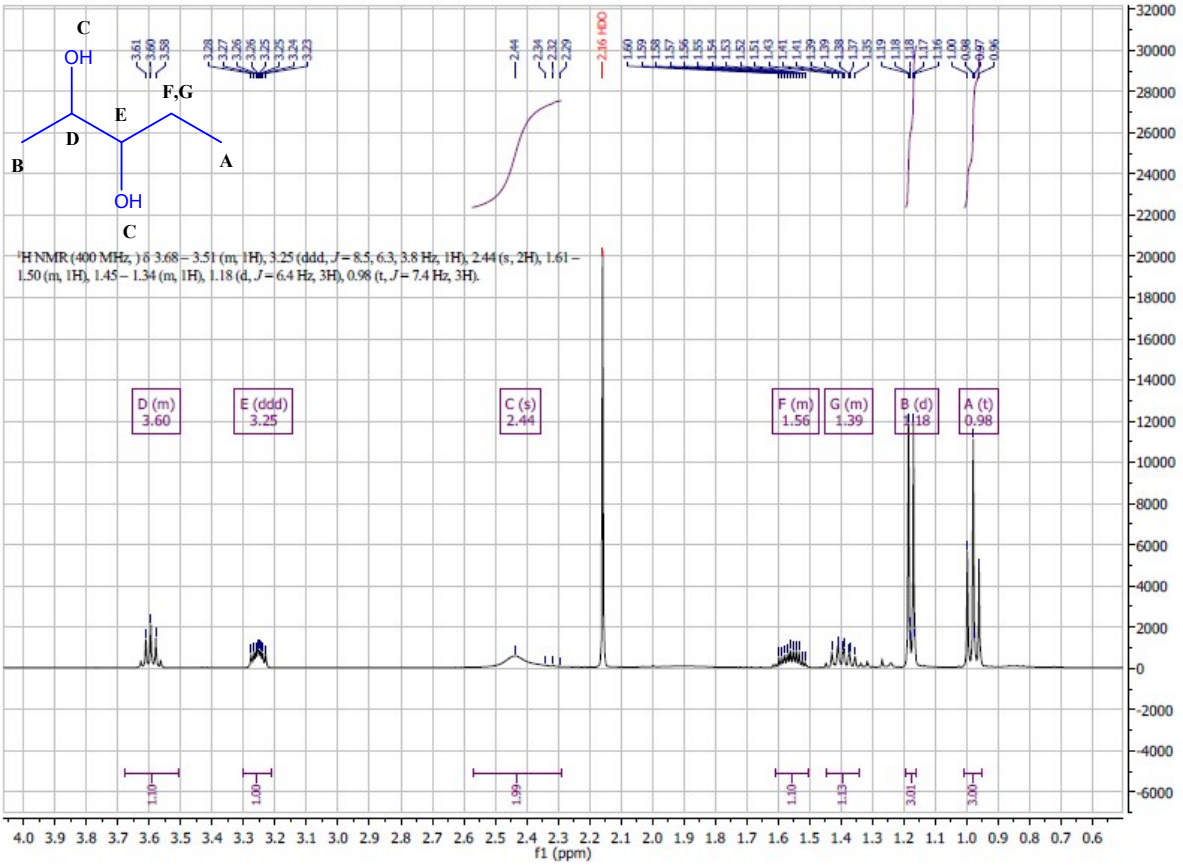
Supplementary Figure 8: GC chromatograms of 2,3-hexanedione (**1b**) standard reduction reactions catalysed by BudC (blue trace, preparative scale), and ADH-380 (green trace). Black trace corresponds to the purified product (*S*)-3-hydroxyhexan-2-one (**3b**). Retention times: internal standard: 6.1 min; (*S*)-3-hydroxyhexan-2-one (**3b**): 8.1 min; putative 2,3-hexanediol (**4b**): 11.5 min.

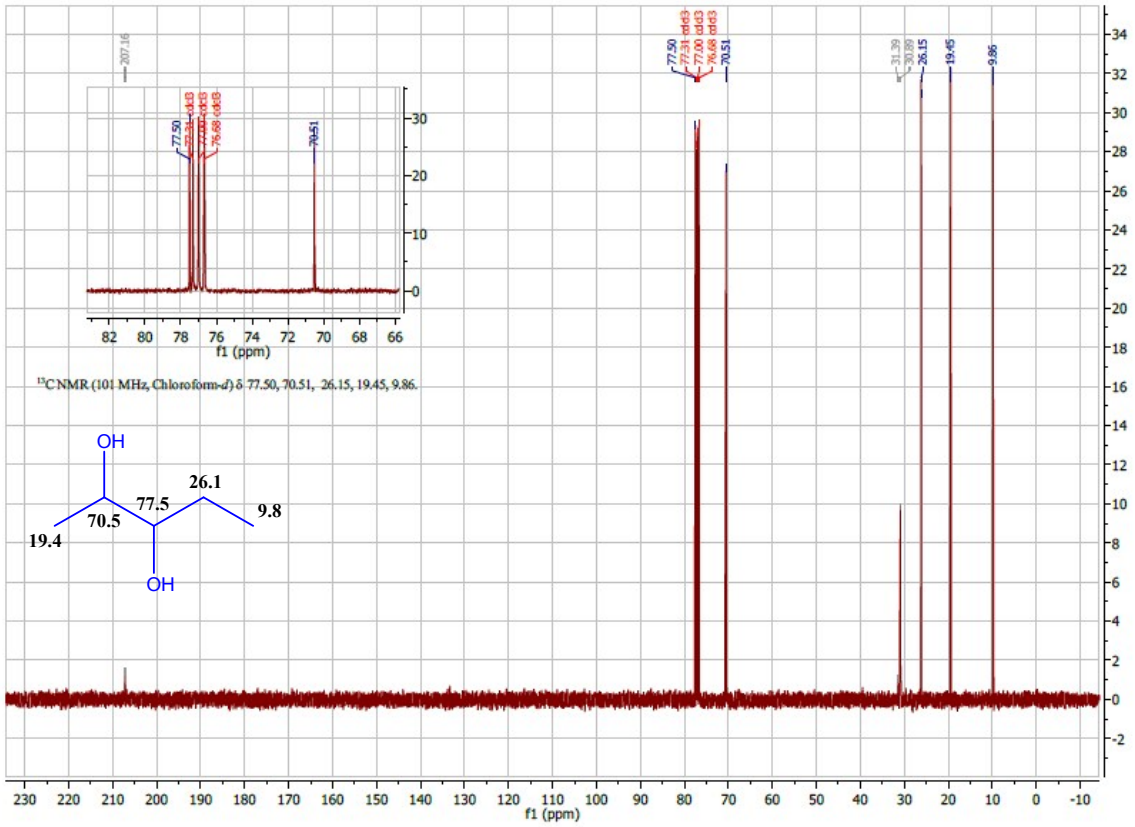


Supplementary Figure 9: GC chromatograms of 3,4-hexanedione (**1c**) standard reduction reactions catalysed by BudC (green and black traces (2h and 24 h reaction, respectively), and ADH-380 (blue trace, 24 h). Red trace corresponds to the blank reaction. Retention times: 3,4-hexanedione (**1c**): 6 min; internal standard: 7.5 min; (*S*)-4-hydroxyhexan-3-one (**2c**): 9.6 min; (3*S*,4*S*)-3,4-hexanediol (**4c**): 12.9 min.

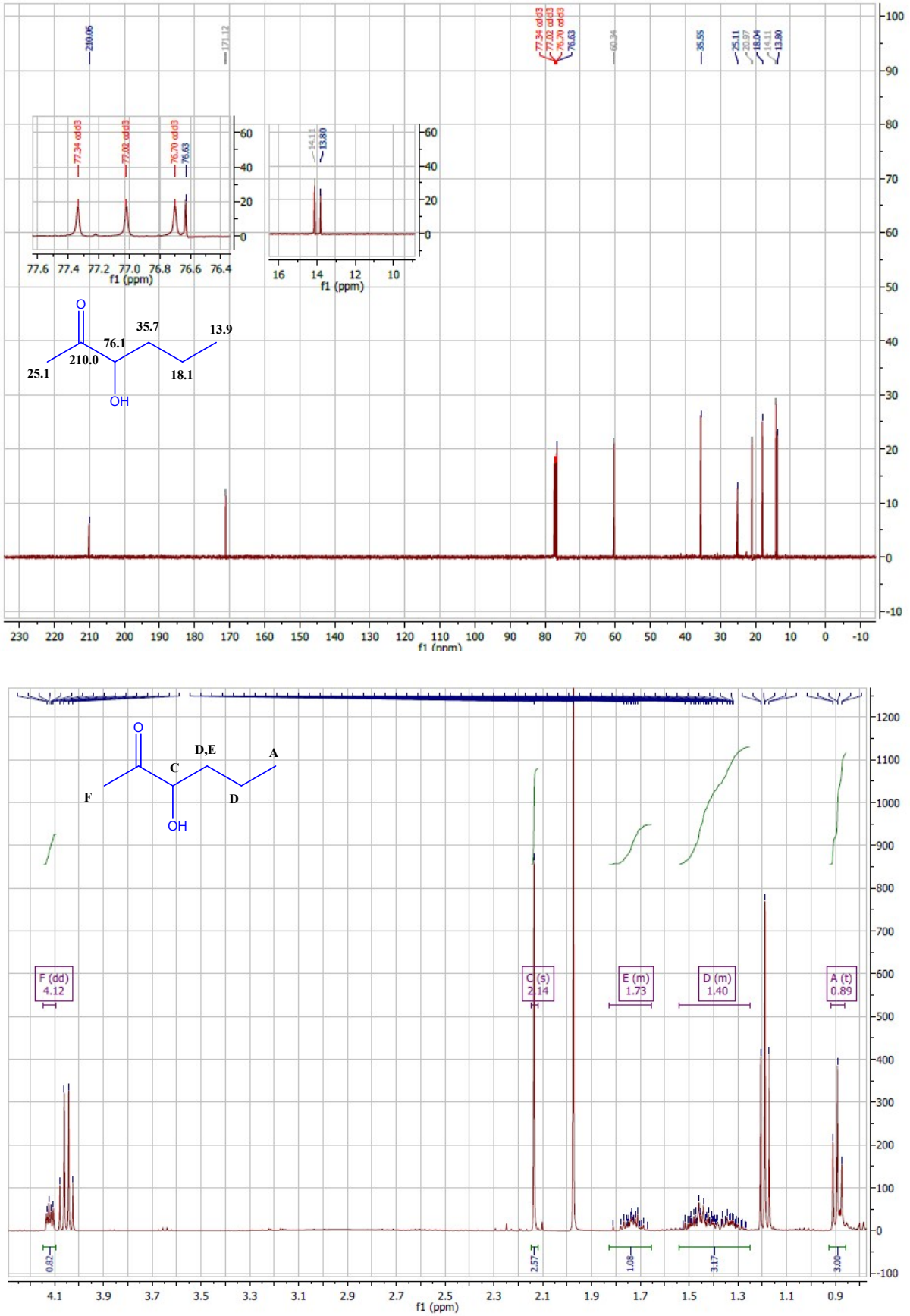


Supplementary Figure 10: GC chromatograms of 2,3-heptanedione (**1d**) standard reduction reactions catalysed by BudC (blue and black traces (2h and 24 h reaction, respectively), and ADH-380 (green trace, 24 h). Retention times: 2,3-heptanedione (**1d**): 7.4 min; internal standard: 7.7 min; (*R*)-2-hydroxyheptan-3-one: 12.0 min; (*S*)-2-hydroxyheptan-3-one (**2d**) : 12.2 min; 3-hydroxy-2-heptanone (**3d**): 12.7 min; (2*S*,3*R*)-2,3-heptanediol (**4d**): 15.8 min.

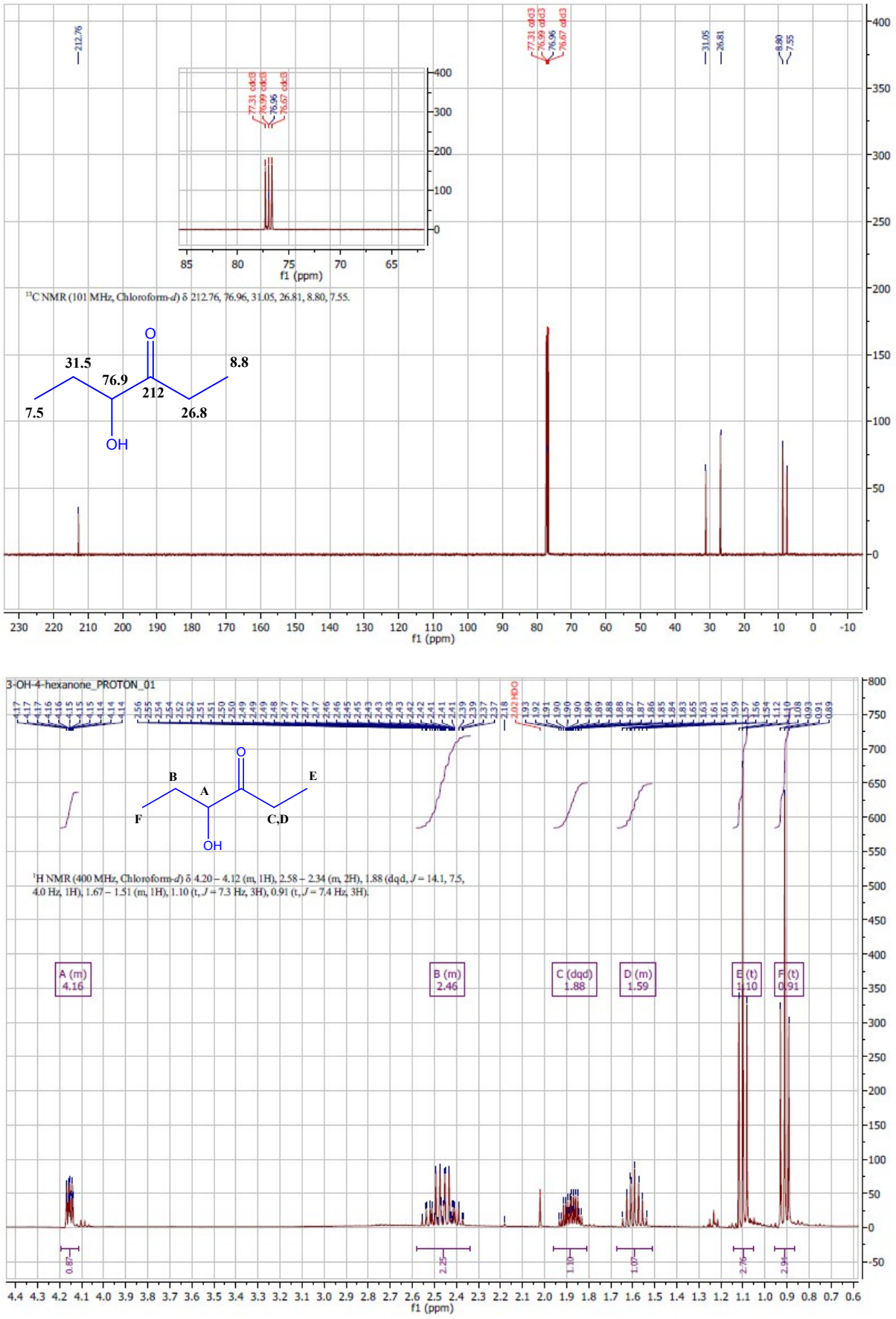




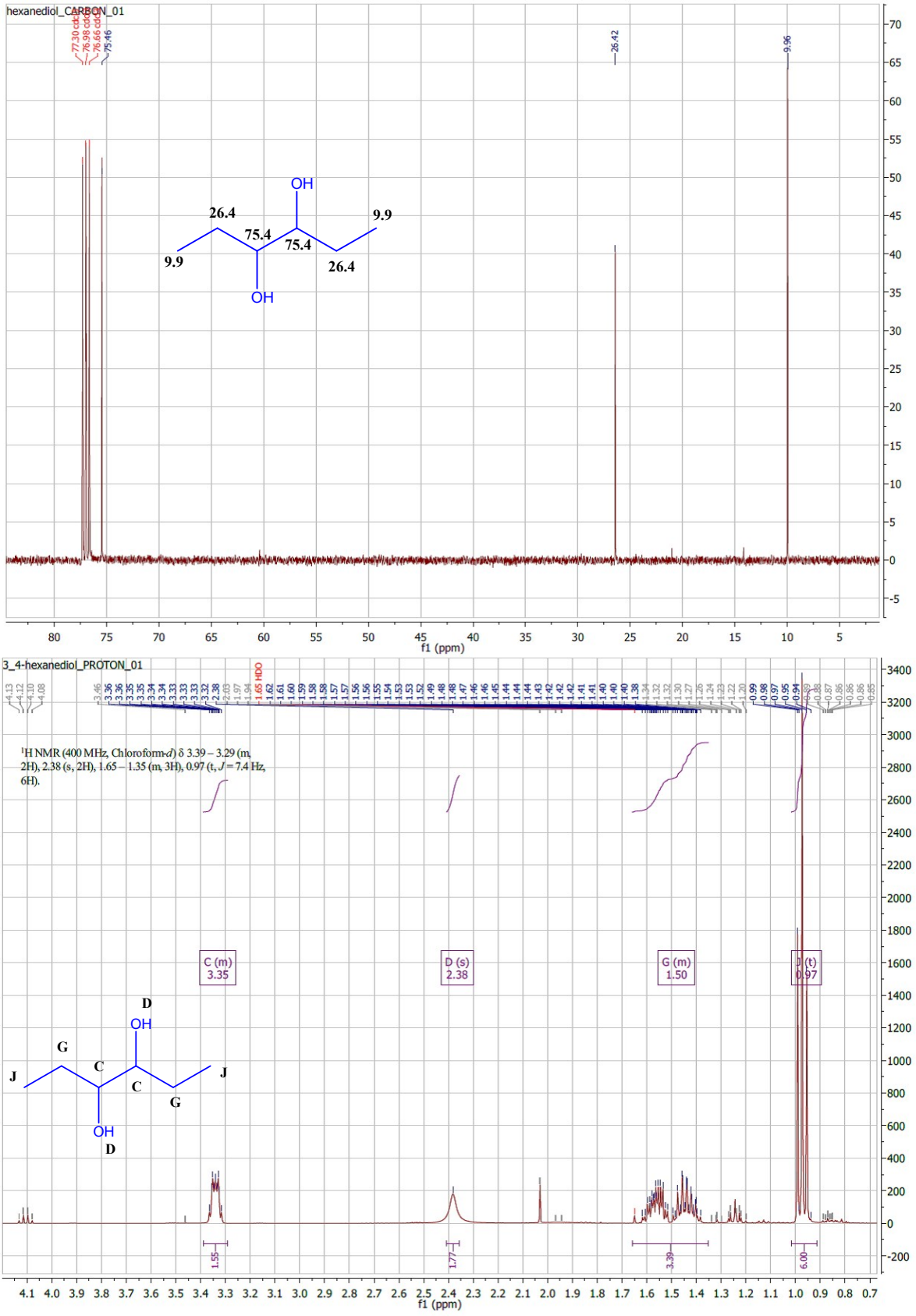
Supplementary Figure 11: ^{13}C and ^{1}H NMR of (*S*,*S*)-2,3-pentanediol (**4a**). Acetone signals were not assigned and marked in grey.



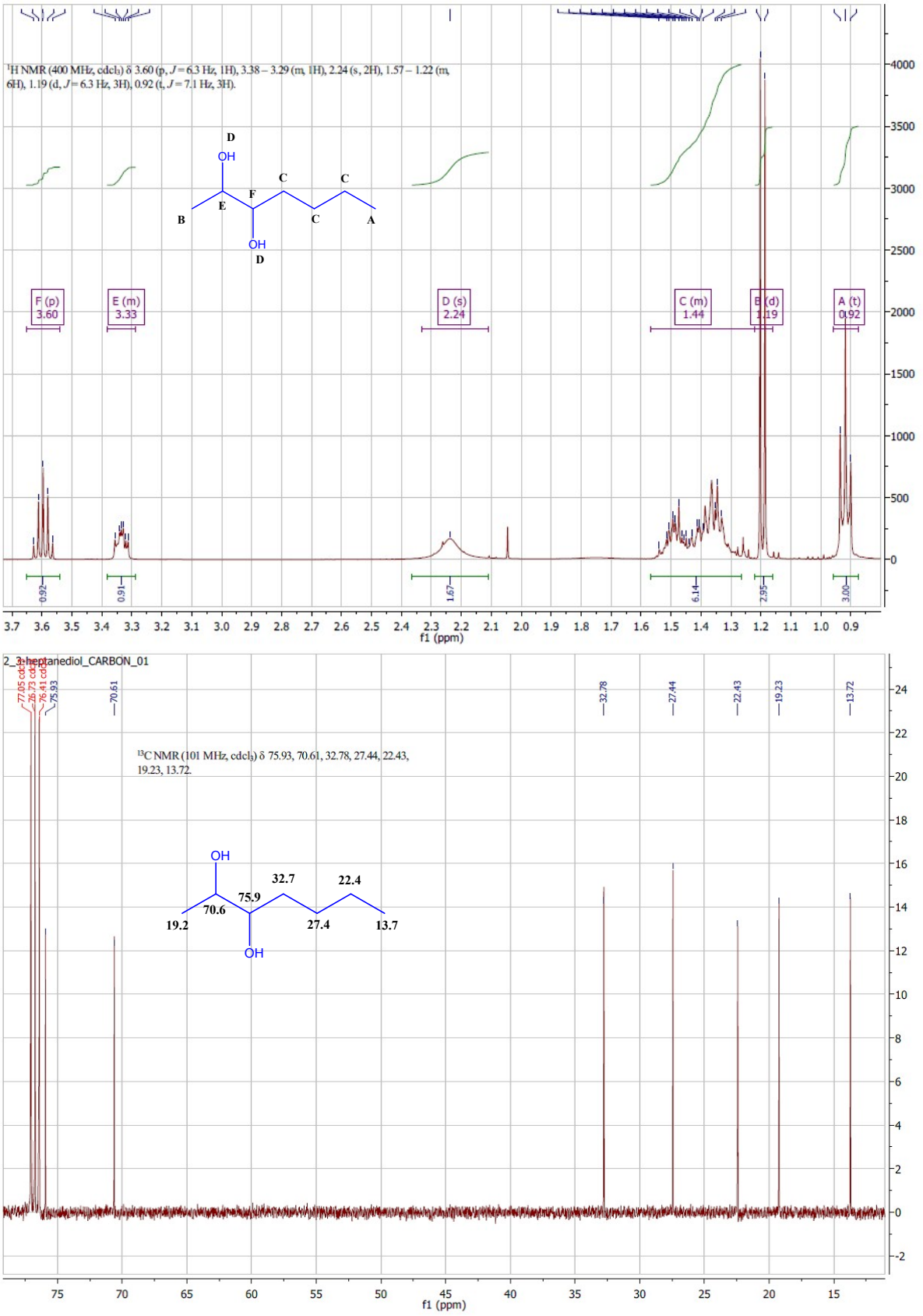
Supplementary Figure 12: ^{13}C and ^{1}H NMR of (*S*)-3-hydroxy-2-hexanone (**3b**). Ethyl acetate signals were not assigned.



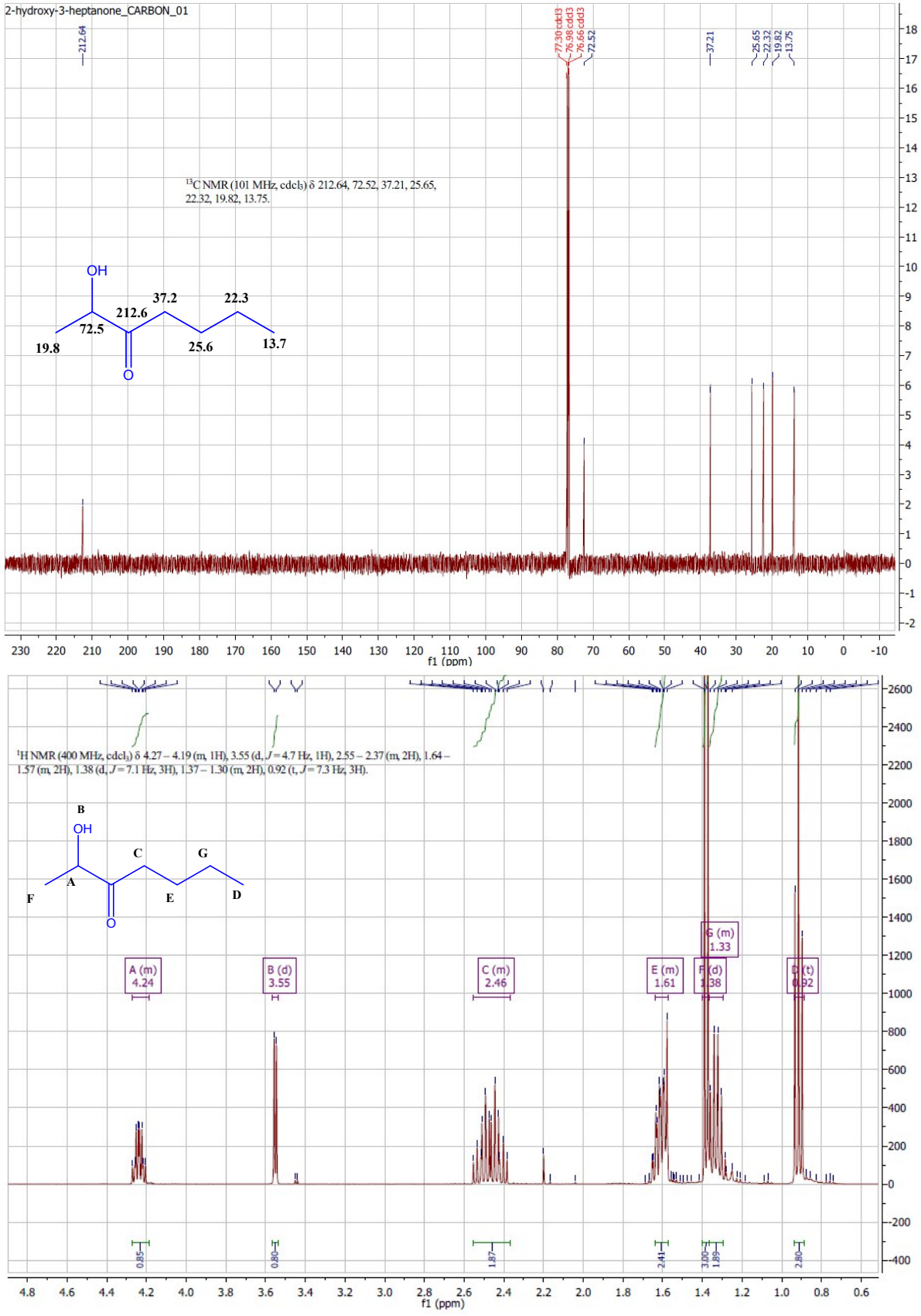
Supplementary Figure 13: ^{13}C and ^{1}H NMR of (*S*)-4-hydroxy-3-hexanone (**2c**).



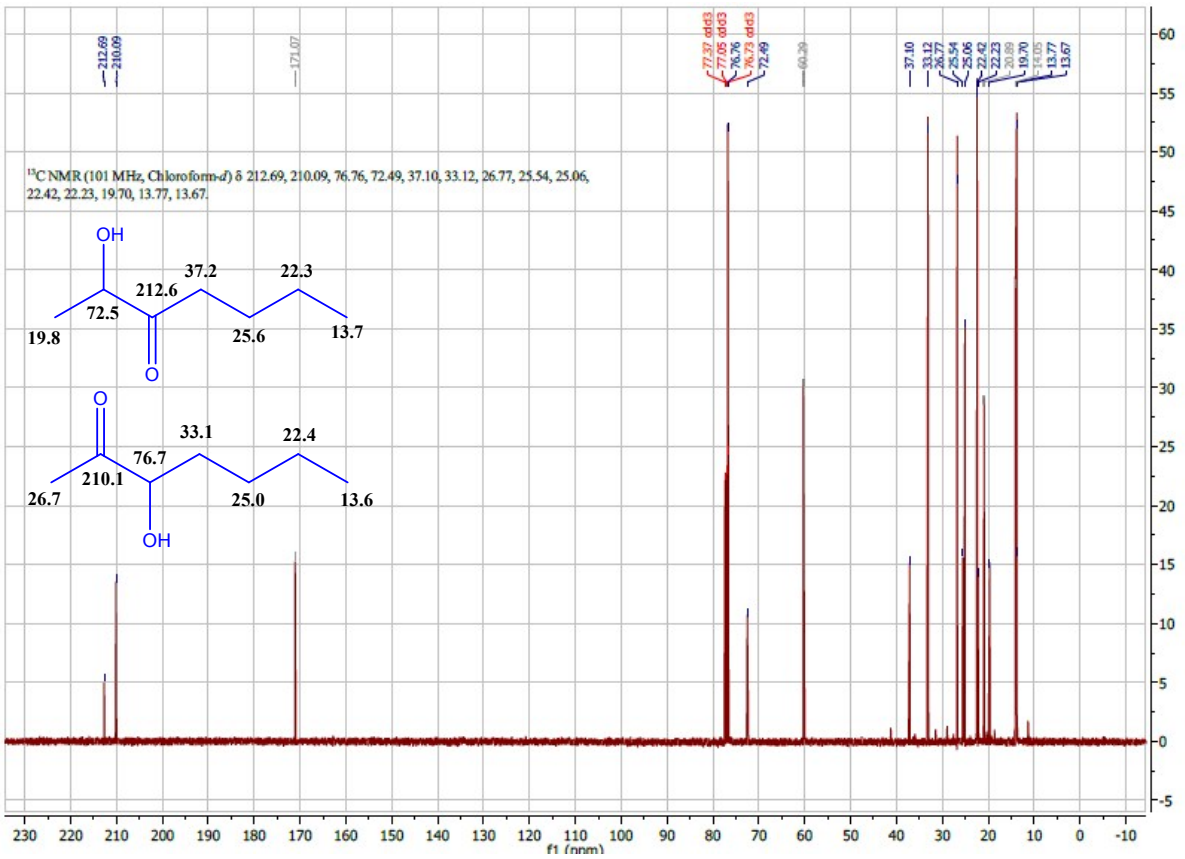
Supplementary Figure 14: ^{13}C and ^{1}H NMR of (*S*,*S*)-3,4-hexanediol (**4c**).



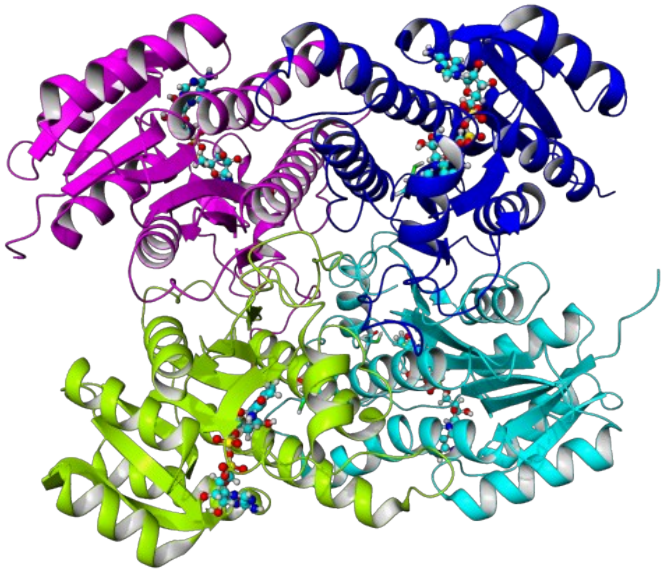
Supplementary Figure 15: ^{13}C and ^{1}H NMR of (*S*,*R*)-2,3-heptanediol (**4d**).



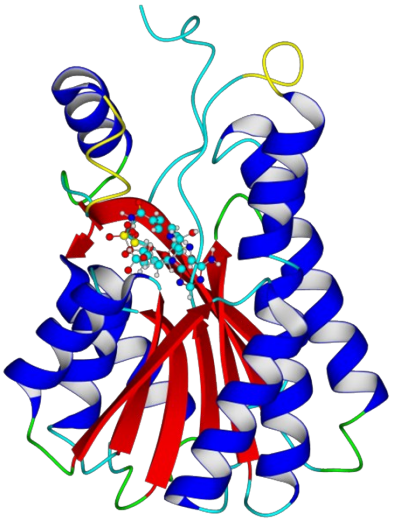
Supplementary Figure 16: ^{13}C and ^{1}H NMR of (*S*)-2-hydroxyheptan-3-one (**2d**).



Supplementary Figure 17:[13]C NMR mixture 2-hydroxy-3-heptanone (**2d**) and 3-hydroxy-2-heptanone (**3d**). Ethyl acetate signals are marked in grey.



Supplementary Figure 18: Homology model of BudC based on the *meso*-2,3-butanediol dehydrogenase from *Klebsiella pneumoniae* (1GEG). The enzyme is a tetramer with NADH (ball and sticks) bound to each active site.



Supplementary Figure 19: Homology model BudC monomer showing the Rossmann fold binding the nicotinamide cofactor.

6. References

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