Supporting Information for:

A biocatalytic method for the chemoselective aerobic oxidation of aldehydes to carboxylic acids

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Table of Contents

1.	Abbreviations	2
2.	General information	2
3.	Expression and purification of recombinant proteins	2
4.	Influence of DTT	3
5.	Temperature profile of ALDH-Bov, ALDH-Ec and PP-ALDH in biocatalytic reactions	5
6.	pH Profile of ALDH-Bov, ALDH-Ec and PP-ALDH in biocatalytic reactions	7
7.	Calculation of response factors between IS and esters	9
8.	Substrate scope	11
9.	Testing the possible enantioselectivity of ALDHs	13
10.	Use of lyophilized cells for biocatalytic reactions (51a and 54a)	14
11.	Lyophilized cells for biocatalytic reactions: Increased substrate concentration (51a)	15
12.	Use of resting *E. coli* cells for biocatalytic reactions (51a and 54a)	16
13.	Testing for the reverse reaction: reduction of carboxylic acid to aldehyde	17
14.	Thermostability measurements	18
15.	Analytical methods	19
16.	NMR spectra of upscaling	21
17.	References	22

1. Abbreviations

ALDH-Bov	Aldehyde dehydrogenase from bovine lens
ALDH-Ec	Aldehyde dehydrogenase from *Escherichia coli*
PP-ALDH	Aldehyde dehydrogenase from *Pseudomonas putida*
NOx	H_2O-forming NADH Oxidase from *Streptococcus mutans*
n.d.	not detected
n.m.	not measured
IS	internal standard

2. General information

All chemicals were purchased in highest grade available without further purification from Sigma Aldrich, TCI chemicals or Alfa Aesar. Nicotinamide cofactor (NAD^+) was purchased from Melford Biolaboratories (Chelsworth, Ipswich, UK).

3. Expression and purification of recombinant proteins

The aldehyde dehydrogenases ALDH-Bov and ALDH-Ec were expressed and purified as described previously.[1]

PP-ALDH[2] was expressed with a N-term His6 tag (pET28b), NOx[3] was expressed with a C-term His6 tag (pET21a). For both proteins *E. coli* BL21 DE3 was used as host organism: 800 mL of LB medium supplemented with ampicillin (100 µg/mL for pET21a) or kanamycin (50 µg/mL for pET28b) were inoculated with 15 mL of an overnight culture. Cells were grown at 37 °C until an OD_{600} of 0.6 – 1 was reached and the expression of the proteins was induced by the addition of IPTG (0.5 mM final concentration). Protein expression was carried out overnight at 25 °C and after harvesting of the cells (4 °C, 4.5 x 10^3 rpm, 15 min), the remaining cell pellet resuspended in Lysis buffer (50 mM KH2PO4, 300 mM NaCl, 10 mM imidazole, pH 8.0). For NOX a tiny spatula tip of FAD was added. Cells were disrupted by ultrasonication. After centrifugation (4 °C, 14 x 10^3 rpm, 1 h) the supernatant was filtered through a 0.45 µm filter and protein purification was performed by Ni-NTA affinity chromatography using pre-packed Ni- NTA HisTrap FF columns (GE Healthcare) according to the manufacturer´s instructions. After loading of the filtered lysate, the column was washed with sufficient amounts of wash buffer (50 mM KH_2PO_4, 300 mM NaCl, 25 mM imidazole, pH 8.0), and bound protein was recovered with elution buffer (50 mM KH_2PO_4, 300 mM NaCl, 200 mM imidazole, pH 8.0). The process of purification was analysed by SDS-PAGE and fractions containing sufficient purified protein were pooled and dialyzed over night against potassium phosphate buffer (50 mM, pH 8,). For NOx, the concentration of flavin saturated protein was estimated by UV-vis spectroscopy at 450 nm by using the characteristic extinction coefficient of free FAD (ε=11300 M^{-1} cm^{-1}). The concentration of PP-ALDH was determined at 280 nm (ε=68600 M^{-1} cm^{-1}).

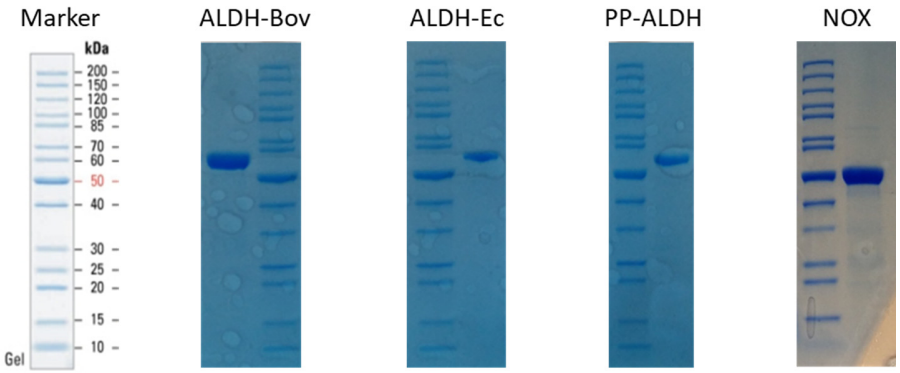


Figure S1. SDS-PAGE of purified enzymes. Marker: PageRuler™ Unstained Protein Ladder (ThermoFisher Scientific).

4. Influence of DTT

Experimental conditions: 1 mL final volume in Eppendorf tubes, buffer = 50 mM Tris/HCl pH 9, 180 rpm on orbital shaker, [**1a**] or [**2a**] = 20 mM, [NAD^+] = 0.5 mM, [ALDH] = 10 µM, [NOx] = 3.3 to 5 µM, reaction time 20 h at 40 °C. Reactions were performed with and without the addition of [DTT] = 100 µg/mL. Moreover, different enzyme charges (ALDH and NOx) have been tested. Extraction with EtOAc under acidic conditions using toluene as IS and derivatization of acids to the methylester with (trimethylsilyl)diazomethane. Conversions were measured by GC-FID (DB-1701).

Table S1. Influence of DTT for the **c**onversion of **1a** to **1b** [%].

ALDH	NOX	DTT	1b [%]
ALDH-BOV (1)	NOx (1)	-	91
ALDH-BOV (2)	NOx (1)	-	67
ALDH-EC (1)	NOx (1)	-	41
ALDH-EC (2)	NOx (1)	-	34
PP-ALDH	NOx (1)	-	16
ALDH-BOV (1)	NOx (1)	+	>99
ALDH-BOV (2)	NOx (1)	+	>99
ALDH-EC (1)	NOx (1)	+	>99
ALDH-EC (2)	NOx (1)	+	>99
PP-ALDH	NOx (1)	+	>99
ALDH-BOV (1)	NOx (2)	-	34
ALDH-BOV (2)	NOx (2)	-	32
ALDH-EC (1)	NOx (2)	-	24
ALDH-EC (2)	NOx (2)	-	18
PP-ALDH	NOx (2)	-	13
ALDH-BOV (1)	NOx (2)	+	>99
ALDH-BOV (2)	NOx (2)	+	>99
ALDH-EC (1)	NOx (2)	+	>99
ALDH-EC (2)	NOx (2)	+	99
PP-ALDH	NOx (2)	+	61
ALDH-BOV (1)	NOx (3)	-	37
ALDH-BOV (2)	NOx (3)	-	29
ALDH-EC (1)	NOx (3)	-	23
ALDH-EC (2)	NOx (3)	-	21
PP-ALDH	NOx (3)	-	12
ALDH-BOV (1)	NOx (3)	+	>99
ALDH-BOV (2)	NOx (3)	+	>99
ALDH-EC (1)	NOx (3)	+	>99
ALDH-EC (2)	NOx (3)	+	99
PP-ALDH	NOx (3)	+	>99

the numbers in brackets indicate different charges of protein

Table S2. Influence of DTT for the **c**onversion of **2a** to **2b** [%].

ALDH	NOX	DTT	2b [%]
ALDH-BOV (1)	NOx (1)	-	24
ALDH-BOV (2)	NOx (1)	-	15
ALDH-EC (1)	NOx (1)	-	18
ALDH-EC (2)	NOx (1)	-	16
PP-ALDH	NOx (1)	-	11
ALDH-BOV (1)	NOx (1)	+	>99
ALDH-BOV (2)	NOx (1)	+	>99
ALDH-EC (1)	NOx (1)	+	>99
ALDH-EC (2)	NOx (1)	+	>99
PP-ALDH	NOx (1)	+	13
ALDH-BOV (1)	NOx (2)	-	32
ALDH-BOV (2)	NOx (2)	-	15
ALDH-EC (1)	NOx (2)	-	15
ALDH-EC (2)	NOx (2)	-	16
PP-ALDH	NOx (2)	-	11
ALDH-BOV (1)	NOx (2)	+	>99
ALDH-BOV (2)	NOx (2)	+	>99
ALDH-EC (1)	NOx (2)	+	>99
ALDH-EC (2)	NOx (2)	+	>99
PP-ALDH	NOx (2)	+	11
ALDH-BOV (1)	NOx (3)	-	13
ALDH-BOV (2)	NOx (3)	-	12
ALDH-EC (1)	NOx (3)	-	15
ALDH-EC (2)	NOx (3)	-	16
PP-ALDH	NOx (3)	-	10
ALDH-BOV (1)	NOx (3)	+	>99
ALDH-BOV (2)	NOx (3)	+	>99
ALDH-EC (1)	NOx (3)	+	>99
ALDH-EC (2)	NOx (3)	+	>99
PP-ALDH	NOx (3)	+	13

the numbers in brackets indicate different charges of protein

5. Temperature profile of ALDH-Bov, ALDH-Ec and PP-ALDH in biocatalytic reactions

Experimental conditions: 1 mL final volume in Eppendorf tubes, buffer = 50 mM Tris/HCl pH 9, 180 rpm on orbital shaker, [**1a**] or [**2a**] = 20 mM, [NAD^+] = 0.5 mM, [ALDH-Bov] = 5 µM for **1a** and 10 µM for **2a**, [ALDH-Ec] = 5 µM, [PP-ALDH] = 20 µM, [NOx] = 5 µM, [DTT] = 100 µg/mL. Extraction with EtOAc under acidic conditions using toluene as IS and derivatization of acids to the methylester with (trimethylsilyl)diazomethane. Conversions were measured by GC-FID (DB-1701).

The progress of the reaction at different temperatures was followed over time and conversions for **1a** and **2a** are summarized in Figure 1 (main paper) and Table S3 and Table S4.

Table S3. Conversion of **1a** [%] to **1b** in 50 mM Tris/HCl pH 9 buffer at different temperatures over time.

enzyme	time [min]	30 °C	40 °C	50 °C
ALDH-Bov (5 µm)	10	15	31	1.5
	20	19	68	15
	30	23	79	17
	60	57	91	92
	90	89	84	95
	120	92	93	93
	180	92	96	93
	240	92	>99	94
ALDH-Ec (5 µM)	10	65	63	25
	20	69	78	41
	30	74	91	59
	60	86	94	88
	90	96	99	94
	120	90	93	81
	180	91	95	94
	240	88	91	94
PP-ALDH (20 µM)	15	11	8.3	15
	30	12	16	25
	60	16	37	55
	90	17	62	73
	120	30	84	88
	180	34	97	94
	240	48	>99	93

Table S4. Conversion of **2a** [%] to **2b** in 50 mM Tris/HCl pH 9 buffer at different temperatures over time.

enzyme	time [min]	30 °C	40 °C	50 °C
ALDH-Bov (10 µM)	10	11	14	11
	20	14	21	11
	30	18	34	12
	60	22	61	83
	90	33	>99	91
	120	41	93	98
	180	67	96	91
	240	88	>99	87
ALDH-Ec (5 µM)	15	68	79	64
	30	95	98	72
	45	96	95	67
	60	64	98	68
	90	91	92	66
	120	81	92	64
	180	99	91	58
	240	91	88	59
PP-ALDH (20 µM)	15	9.6	8.2	11
	30	9.1	9.5	12
	60	9.8	9.7	13
	90	9.0	9.8	13
	120	9.1	10	11
	180	11	10	13
	240	9.4	10	14

6. pH Profile of ALDH-Bov, ALDH-Ec and PP-ALDH in biocatalytic reactions

Experimental conditions: 1 mL final volume in Eppendorf tubes, buffer strength = 50 mM (Gly/NaOH buffer = 50 mM glycine titrated with NaOH to pH 9) T = 40 °C, 180 rpm on orbital shaker, [**1a**] or [**2a**] = 20 mM, [NAD^+] = 0.5 mM, [ALDH-Bov] = 5 µM for **1a** and 10 µM for **2a**, [ALDH-Ec] = 5 µM, [PP-ALDH] = 20 µM, [NOx] = 5 µM, [DTT] = 100 µg/mL. Extraction with EtOAc under acidic conditions using toluene as IS and derivatization of acids to the methylester with (trimethylsilyl)diazomethane. Conversions were measured by GC-FID (DB-1701).

The progress of the reaction using various buffer species and pH values was followed over time and conversions for **1a** and **2a** are summarized in Figure 2 (main paper) and Table S5 and Table S6.

Table S5. Conversion of **1a** [%] to **1b** using different types of buffers over time.

enzyme	time [min]	Tris/HCl pH 7	Tris/HCl pH 8	Tris/HCl pH 9	KPi pH 8	Gly/NaOH pH 9	Tris/HCl pH 8.5	KPi pH 8.5
ALDH-Bov (5 µM)	15	17	22	48	27	24	n.m.	n.m.
	30	24	47	79	60	40	n.m.	n.m.
	45	30	83	86	91	53	n.m.	n.m.
	60	36	96	91	91	68	n.m.	n.m.
	90	37	95	84	90	89	n.m.	n.m.
	120	43	97	93	83	90	n.m.	n.m.
	180	40	97	97	>99	88	n.m.	n.m.
	240	41	96	>99	93	85	n.m.	n.m.
ALDH-Ec (10 µM)	15	15	78	71	87	41	n.m.	n.m.
	30	15	>99	87	95	46	n.m.	n.m.
	45	14	93	90	95	45	n.m.	n.m.
	60	15	95	92	91	44	n.m.	n.m.
	90	14	94	93	93	42	n.m.	n.m.
	120	15	94	94	94	43	n.m.	n.m.
	180	15	>99	90	97	44	n.m.	n.m.
	240	14	92	91	98	44	n.m.	n.m.
PP-ALDH (20 µM)	15	15	24	8.3	27	17	18	22
	30	19	40	16	45	18	33	40
	45	21	53	25	62	20	47	54
	60	25	66	37	78	20	58	72
	90	27	83	62	89	25	77	87
	120	31	95	84	92	26	90	92
	180	37	97	97	94	35	88	87
	240	38	92	>99	>99	30	84	86

n.m. not measured

Table S6. Conversion of **2a** [%] to **2b** using different types of buffers over time.

enzyme	time [min]	Tris/HCl pH 7	Tris/HCl pH 8	Tris/HCl pH 9	KPi pH 8	Gly/NaOH pH 9	Tris/HCl pH 8.5	KPi pH 8.5
ALDH-Bov (10 µM)	15	16	14	17	33	13	n.m.	n.m.
	30	22	33	34	40	26	n.m.	n.m.
	45	24	62	43	74	43	n.m.	n.m.
	60	27	96	61	96	53	n.m.	n.m.
	90	31	83	>99	99	60	n.m.	n.m.
	120	29	93	93	>99	62	n.m.	n.m.
	180	31	97	96	88	64	n.m.	n.m.
	240	29	92	>99	96	64	n.m.	n.m.
ALDH-Ec (5 µM)	15	9.0	62	79	67	39	n.m.	n.m.
	30	9.6	91	98	97	41	n.m.	n.m.
	45	8.0	90	95	95	39	n.m.	n.m.
	60	8.9	90	98	98	39	n.m.	n.m.
	90	8.8	89	92	94	38	n.m.	n.m.
	120	13	90	92	95	40	n.m.	n.m.
	180	17	83	91	90	38	n.m.	n.m.
	240	15	79	88	90	35	n.m.	n.m.
PP-ALDH (20 µM)	15	11	24	8.2	23	10	15	22
	30	17	38	9.5	39	10	20	36
	45	20	52	9.5	55	11	24	46
	60	22	65	9.7	74	14	28	63
	90	26	84	9.8	93	11	27	83
	120	25	92	10	97	11	31	76
	180	26	96	10	102	11	51	91
	240	26	87	10	99	10	25	85

n.m. not measured

7. Calculation of response factors between IS and esters

Experimental conditions:

20 mM of reference acid **1b-60b** were pipetted into EtOAc containing 20 mM IS (final volume 1 mL, in Eppendorf tubes). After dilution to 5 mM and derivatization to the methylester, the area of IS and ester were determined by GC-FID.

Additionally, 20 mM of acid were incubated in 50 mM KPi pH 8.5 buffer for 2 h at 40 °C, extracted with EtOAc containing IS, derivatized and peak areas were determined by GC-FID. For enhancing the extraction of **51b**, the buffer phase was saturated with NaCl prior the addition of EtOAc.

The response factor was calculated from the ratio peak are ester/peak area IS (samples that were extracted), and was used to determine the conversion of the substrates by the ALDHs. Only substrates were further tested with the ALDHs, that could get extracted in sufficient amounts (>75%). Measurements were done in duplicates.

The acids were pipetted as liquids or as stock solutions of 1M or 2M in DMSO, depending on their solubility.

Table S7. Response factors of acids (ester) and IS

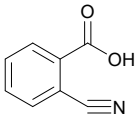
acid	DMSO stock	Peak Area IS EtOAc		Peak Area IS extracted		Peak Area ester EtOAc		Peak Area ester extracted		response factor		substrate extractable
		m1	m2	m1	m2	m1	m2	m1	m2	EtOAc	extracted	
1b	-	1189	1195	1410	1517	993	1020	1155	1183	0.8444	0.7995	95%
2b	2 M	991	994	1181	1182	1027	1017	1157	1161	1.0297	0.9810	95%
3b	-	1147	1129	1352	1332	695	664	737	653	0.5970	0.5177	87%
4b	-	1103	1151	1328	1341	807	806	760	737	0.7160	0.5609	78%
5b	-	1155	1199	1301	1292	771	800	674	665	0.6674	0.5164	77%
6b	-	1151	1146	1277	1278	928	917	992	987	0.8032	0.7746	96%
7b	-	1144	1046	1267	1279	958	958	966	990	0.8766	0.7682	88%
8b	2 M	1112	1021	1307	1264	946	858	1044	973	0.8455	0.7843	93%
9b	-	1124	1087	1272	1265	1093	1077	1149	1159	0.9816	0.9098	93%
10b	-	1138	1146	1294	1286	1241	1245	1328	1301	1.0884	1.0190	94%
11b	-	1159	1130	1291	1293	1413	1363	1441	1451	1.2127	1.1192	92%
12b	2 M	1008	871	1167	1160	1306	1292	1362	1437	1.3895	1.2029	87%
13b	-	1152	1110	1296	1293	1115	1097	1104	1097	0.9781	0.8501	87%
14b	2 M	989	980	1171	1147	1270	1218	1403	1357	1.2635	1.1906	94%
15b	2 M	960	998	1176	1167	1250	1242	1405	1405	1.2733	1.1993	94%
16b	1 M	1073	1019	1217	1204	1077	1085	1264	1249	1.0342	1.0380	100%
17b	2 M	954	993	1168	1185	1140	1153	1311	1327	1.1780	1.1211	95%
18b	2 M	1016	998	1180	1198	1150	1130	1274	1289	1.1321	1.0778	95%
19b	1 M	1019	1003	1153	1175	1174	1154	1281	1330	1.1513	1.1215	97%
20b	1 M	945	947	1141	1151	1251	1255	1141	1151	1.3245	1.2714	96%
21b	2 M	1017	1005	1128	1143	1057	1020	1150	1175	1.0271	1.0238	100%
22b	2 M	995	984	1202	1191	1026	1000	1189	1165	1.0237	0.9837	96%
23b	2 M	1004	953	1187	1181	1034	1034	1174	1162	1.0574	0.9865	93%
24a	1 M	905	944	1145	1154	904	922	1048	1086	0.9823	0.9282	95%
25b	2 M	995	1008	1163	1163	1002	988	1130	1175	0.9936	0.9910	100%
26b	2 M	978	985	1200	1189	1010	1013	1157	1160	1.0306	0.9699	94%
27b	1 M	990	1002	1163	1159	1002	1000	1109	1121	1.0051	0.9604	96%

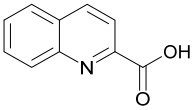
Table S8. Response factors of acids (ester) and IS

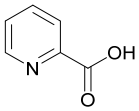
acid	DMSO stock	Peak Area IS EtOAc		Peak Area IS extracted		Peak Area ester EtOAc		Peak Area ester extracted		response factor		substrate extractable
		m1	m2	m1	m2	m1	m2	m1	m2	EtOAc	extracted	
28b	1 M	932	1006	1252	1303	923	902	1099	1158	0.9435	0.8833	94%
29b	2 M	978	974	1179	1180	975	986	1113	1106	1.0046	0.9407	94%
30b	2 M	994	974	1176	1181	966	965	1108	1095	0.9813	0.9347	95%
31b	1 M	1004	957	1183	1175	933	937	1088	1086	0.9542	0.9220	97%
32b	2 M	991	980	1195	1196	1046	1032	1172	1194	1.0543	0.9895	94%
33b	2 M	998	970	1193	1175	1003	988	1134	1132	1.0118	0.9570	95%
34b	1 M	902	899	1034	1038	785	779	881	910	0.8684	0.8644	100%
35b	1 M	899	868	1045	1055	752	748	854	871	0.8491	0.8214	97%
36b	1 M	1152	1100	1277	1208	878	845	1007	967	0.7652	0.7945	104%
37b	1 M	862	864	1048	1056	898	905	1011	1015	1.0446	0.9629	92%
38b	1 M	904	912	1046	1056	864	876	1003	1028	0.9581	0.9662	101%
39b	2 M	1004	1002	1163	1179	983	986	1120	1126	0.9816	0.9590	98%
40b	1M	948	970	1169	1162	869	867	1025	977	0.9052	0.8588	95%
41b	1 M	1008	1055	1178	1178	1016	1190	1145	1148	1.0679	0.9733	91%
42b	1 M	997	976	1175	1190	1014	1014	1128	1163	1.0280	0.9687	94%
43b	0.5 M	1080	1085	1215	1226	1046	1033	1172	1185	0.9603	0.9656	101%
44b	1 M	1083	1064	1214	1219	1013	1006	1155	1155	0.9404	0.9494	101%
45b	1 M	1087	1097	1352	1313	960	957	1045	1089	0.8778	0.8012	91%
46b	2 M	980	983	1175	1182	1198	1151	1264	1278	1.1967	1.0785	90%
47b	1 M	963	1025	1163	1173	886	907	995	994	0.9025	0.8515	94%
48b	1 M	1098	1117	1310	1294	753	758	861	869	0.6822	0.6644	97%
49b	1 M	1122	1150	1300	1322	632	651	676	672	0.5647	0.5142	91%
50b	2 M	1136	1135	1300	1295	637	624	686	678	0.5553	0.5256	95%
51b	1 M	961	942	1137	958	486	499	484	396	0.5177	0.4195	81%[a]
52b	2 M	1114	1168	1269	1265	752	758	848	860	0.6620	0.6740	102%
53b	1 M	953	983	1168	1166	742	726	847	875	0.7586	0.7378	97%
54b	2 M	1103	1109	1267	1264	720	716	814	835	0.6492	0.6515	100%
55b	2 M	1096	1138	1294	1318	1136	1140	1316	1343	1.0191	1.0180	100%
56b	1 M	1131	1147	1308	1307	1248	1265	1460	1492	1.1032	1.1289	102%
57b	1 M	1088	1113	1260	1272	1337	1340	1529	1555	1.2164	1.2180	100%
58b	1 M	1103	1128	1273	1290	1473	1451	1695	1738	1.3109	1.3394	102%
59b	1 M	1106	1132	1304	1275	1048	1053	1218	1209	0.9389	0.9411	100%
60b	1M	954	957	1169	1180	1305	1329	1525	1579	1.3783	1.3213	96%

[a]the extraction could be improved by the addition of NaCl to the acidified buffer phase prior the extraction with EtOAc.

Substrates that could not get extracted in sufficient amounts were:







8. Substrate scope

Following tables show the full dataset of the substrate scope. Experimental details were performed as described in the main paper. Additionally, blank reactions were performed. Blank A: incubation of substrate in buffer, Blank B: contained buffer, substrate, NAD^+, NOx and DTT (no ALDH was added).

Table S9. Conversions to acids [%] after 4h and 24 h in 50 mM KPi buffer pH 8.5 (40 °C). [substrate = 20 mM].

enzyme	substrate	4 h [%]	24 h [%]	substrate	4 h [%]	24 h [%]	substrate	4 h [%]	24 h [%]
Bov	**3a**	65	67	**12a**	74	77	**21a**	>99	>99
Ec		66	76		75	78		>99	98
PP		67	72		73	80		16	13
Blank A		1	3		7	7		11	12
Blank B		1	2		6	6		10	11
Bov	**4a**	>99	>99	**13a**	>99	>99	**22a**	>99	>99
Ec		>99	>99		>99	>99		>99	>99
PP		>99	>99		>99	>99		13	11
Blank A		2	2		10	11		8	8
Blank B		2	3		8	9		8	8
Bov	**5a**	>99	>99	**14a**	77	83	**23a**	>99	>99
Ec		>99	>99		78	85		>99	>99
PP		>99	>99		76	79		17	5
Blank A		4	4		5	6		4	4
Blank B		4	4		5	5		4	4
Bov	**1a**	95	>99	**15a**	6	6	**24a**	>99	67
Ec		94	>99		24	25		>99	96
PP		95	98		5	5		3	3
Blank A		7	9		5	9		2	3
Blank B		5	7		4	6		2	2
Bov	**6a**	61	69	**16a**	4	4	**25a**	10	11
Ec		92	90		5	5		10	11
PP		11	64		<1	<1		5	5
Blank A		3	3		n.d.	n.d.		5	6
Blank B		3	3		n.d.	n.d.		4	5
Bov	**7a**	72	76	**2a**	>99	>99	**26a**	94	>99
Ec		69	76		>99	>99		>99	>99
PP		68	70		21.4	>99		20	18.1
Blank A		19	19		12	13		4	5
Blank B		20	21		11	12		3	3
Bov	**8a**	10	9	**17a**	51	49	**27a**	>99	>99
Ec		91	92		25	28		>99	>99
PP		66	94		1	1		5	7
Blank A		4	5		4	1		2	2
Blank B		4	5		3	1		2	2
Bov	**9a**	53	60	**18a**	>99	>99	**28a**	n.d.	n.d.
Ec		51	54		25	18		n.d.	n.d.
PP		51	55		2	2		n.d.	n.d.
Blank A		27	27		2	2		n.d.	n.d.
Blank B		5	3		2	2		n.d.	n.d.
Bov	**10a**	61	70	**19a**	>99	>99	**29a**	14	15
Ec		58	62		>99	90		8	8
PP		57	61		3	3		7	7
Blank A		8	9		3	3		8	8
Blank B		7	7		3	3		7	7
Bov	**11a**	66	75	**20a**	2	1	**30a**	97	96
Ec		65	69		2	1		>99	97
PP		64	67		1	1		14	6
Blank A		6	6		2	2		5	5
Blank B		6	6		2	1		5	4

n.d. not detected

Table S10. Conversions to acids [%] after 4h and 24 h in 50 mM KPi buffer pH 8.5 (40 °C). [substrate = 20 mM].

enzyme	substrate	4 h [%]	24 h [%]	substrate	4 h [%]	24 h [%]	substrate	4 h [%]	24 h [%]
Bov	**31a**	>99	>99	**41a**	95	95	**51a**	90	88
Ec		99	>99		1	1		89	91
PP		8	6		n.d.	n.d.		25	11
Blank A		1	2		n.d.	n.d.		n.d.	n.d.
Blank B		1	1		n.d.	n.d.		n.d.	n.d.
Bov	**32a**	94	90	**42a**	91	90	**52a**	>99	>99
Ec		93	90		7	8		>99	>99
PP		92	90		4	4		6	4
Blank A		1	1		2	4		n.d.	n.d.
Blank B		1	1		2	3		n.d.	n.d.
Bov	**33a**	98	94	**43a**	84	>99	**53a**	99	91
Ec		96	93		15	16		>99	93
PP		95	96		3	3		4	4
Blank A		<1	1		1	1		3	3
Blank B		<1	1		1	2		3	3
Bov	**34a**	>99	>99	**44a**	95	>99	**54a**	>99	>99
Ec		1	2		2	3		>99	>99
PP		<1	<1		1	2		3	3
Blank A		<1	<1		1	2		1	1
Blank B		<1	<1		1	2		1	1
Bov	**35a**	>99	>99	**45a**	30	57	**55a**	11	11
Ec		20	14		1	1		7	8
PP		1	<1		n.d.	n.d.		1	1
Blank A		1	n.d.		n.d.	n.d.		n.d.	n.d.
Blank B		n.d.	n.d.		n.d.	n.d.		n.d.	n.d.
Bov	**36a**	93	96	**46a**	2	2	**56a**	49	51
Ec		6	5		2	2		7	8
PP		1	2		2	2		n.d.	n.d.
Blank A		n.d.	n.d.		2	2		n.d.	n.d.
Blank B		n.d.	n.d.		2	2		n.d.	n.d.
Bov	**37a**	10	4	**47a**	58	>99	**57a**	67	48
Ec		2	2		>99	>99		<1	<1
PP		1	1		18	16		n.d.	n.d.
Blank A		1	1		9	9		n.d.	n.d.
Blank B		1	1		3	4		n.d.	n.d.
Bov	**38a**	>99	97	**48a**	34	40	**58a**	98	>99
Ec		43	49		>99	>99		13	17
PP		n.d.	n.d.		11	13		<1	n.d..
Blank A		n.d.	n.d.		1	1		n.d.	n.d.
Blank B		n.d.	n.d.		1	1		n.d.	n.d.
Bov	**39a**	>99	97	**49a**	>99	96	**59a**	1	3
Ec		10	12		>99	98		n.d.	n.d.
PP		<1	<1		4	4		n.d.	n.d.
Blank A		<1	<1		1	1		n.d.	n.d.
Blank B		<1	<1		1	1		n.d.	n.d.
Bov	**40a**	2	2	**50a**	>99	>99	**60a**	67	94
Ec		n.d.	n.d.		>99	>99		3	3
PP		n.d.	n.d.		>99	>99		2	2
Blank A		n.d.	n.d.		n.d.	n.d.		2	2
Blank B		n.d.	n.d.		n.d.	n.d.		2	2
Bov	**61a**	25[a]	27[a]						
Ec		27[a]	26[a]						
PP		25[a]	27[a]						
Blank A		1	1						
Blank B		1	1						

n.d. not detected; [a] for ALDH-Bov and ALDH-Ec racemic ester was measured, ALDH-PP gave a slight preference for the *R* enantiomer (*ee* of 16)

9. Testing the possible enantioselectivity of ALDHs

Experimental conditions: 1 mL final volume in Eppendorf tubes, buffer = 50 mM KPi pH 8.5, 180 rpm on orbital shaker, T = 40 °C, [**1a**] = 20 mM, [NAD^+] = 0.5 mM, [ALDH-Bov] = 5 µM, [ALDH-Ec] = 10 µM, [PP-ALDH] = 20 µM, [DTT] = 100 µg/mL, [NOx] = 5 µM.

The reactions were stopped at different time points (5 min, 10 min, 15 min, 30 min and 60 min), 400 µL were extracted under basic conditions using toluene as IS and the *ee* of the aldehyde was analyzed by GC-FID (Restek-Rt-βDEXsa). Other 400 µL of the reaction were extracted under acidic conditions using toluene as IS and the acid was derivatized to the methylester with (trimethylsilyl)diazomethane. Conversions and *ee* of the ester were measured by GC-FID (DB-1701 and Restek-Rt-βDEXsm).

Table S11. Biocatalytic oxidation of substrate **1a** monitored over the time. Conversion as well as enantiomeric excess of substrate and products were determined.

enzyme	time [min]	*ee* (*S*)-1a [%]	1b [%]	*ee* (*R*)-1b [%]
ALDH-Bov	5	1.7	10	11.0
	10	3.0	13	17.0
	15	6.4	22	21.9
	30	25.1	64	18.0
	60	n.m.	98	*rac*
ALDH-Ec	5	44.2	36	43.4
	10	83.8	66	31.5
	15	>99.9	90	4.4
	30	n.m.	97	*rac*
	60	n.m.	95	*rac*
PP-ALDH	5	0.2	6	*rac*
	10	0.3	6	2.1
	15	0.7	8	5.4
	30	2.4	13	13.5
	60	9.1	31	20

10. Use of lyophilized cells for biocatalytic reactions (51a and 54a)

The ALDH pellets (Bov and Ec) obtained from protein expression were resuspended in KPi buffer (50 mM, pH 8.5) and freeze dried. Additionally *E. coli* BL21 DE3 cells containing empty pET28b plasmid were also tested.

Experimental conditions: Final volume 1 mL in Eppendorf tubes; lyophilized ALDH cells (10 mg/mL) were rehydrated in KPi buffer (50 mM pH 8.5) containing DTT (100 µg/mL). In some reactions, NOx (5 µM, in purified form) and/or NAD^+ (0.5 mM) were added. The reactions were started by the addition of substrate **51a** or **54a** (20 mM, added as 1 M stock solutions in DMSO) and incubated on an orbital shaker at 170 rpm for 24 h at 40°C. The reactions were stopped by the acidic extraction with EtOAc, samples derivatized and conversions measured by GC-FID. Reactions were performed in duplicate.

Table S12. Conversion [%] to **51b** using lyophilized whole cells.

enzyme	NOx	NAD^+	DTT	conversion [%]
ALDH-Bov	+	+	+	79.1 ± 0.4
	+	-	+	80.5 ± 1.2
	-	+	+	79.3 ± 0.7
	-	-	+	79.0 ± 1.4
	-	-	-	78.7 ± 0.2
ALDH-Ec	+	+	+	80.1 ± 1.6
	+	-	+	81.3 ± 0.9
	-	+	+	79.3 ± 1.2
	-	-	+	78.8 ± 0.0
	-	-	-	79.5 ± 0.7
empty *E. coli*	+	+	+	35.6 ± 1.1
	+	-	+	37.3 ± 0.1
	-	+	+	36.1 ± 0.2
	-	-	+	36.1 ± 1.1
	-	-	-	36.5 ± 0.3

Table S13. Conversion [%] to **54b** using lyophilized whole cells.

enzyme	NOx	NAD^+	DTT	conversion [%]
ALDH-Bov	+	+	+	86.9 ± 1.1
	+	-	+	46.3 ± 4.2
	-	+	+	42.6± 28
	-	-	+	15.5 ± 0.3
	-	-	-	13.0 ± 0.6
ALDH-Ec	+	+	+	87.8 ± 0.6
	+	-	+	89.0 ± 0.9
	-	+	+	88.7 ± 0.4
	-	-	+	25.9 ± 5.1
	-	-	-	37.8 ± 8.1
empty *E. coli*	+	+	+	8.1 ± 0.2
	+	-	+	5.8 ± 0.0
	-	+	+	8.4 ± 0.1
	-	-	+	6.0 ± 0.1
	-	-	-	5.9 ± 0.1

11. Lyophilized cells for biocatalytic reactions: Increased substrate concentration (51a)

To obtain a buffering of higher concentrations of acids produced, 200 mM of KPi buffer (pH 8.5) was used.

Experimental conditions: Final volume 1 mL in Eppendorf tubes; lyophilized ALDH cells (10 mg/mL) were rehydrated in KPi buffer (200 mM pH 8.5) containing DTT (100 µg/mL). Reactions were performed with and without NOx (5 µM, in purified form) and NAD^+ (0.5 mM). The reactions were started by the addition of substrate **51a** (20-100 mM, added as 5 M stock solutions in DMSO) and incubated on an orbital shaker at 170 rpm for 24 h at 40°C. The reactions were stopped by the acidic extraction with EtOAc, samples derivatized and conversions measured by GC-FID. Reactions were performed in duplicate.

Table S14. Conversion [% and mM] to **51b** using lyophilized cells and increased substrate concentration with addition of NOx and NAD^+.

enzyme	NOx, NAD^+	substrate [mM]	conversion [%]	conversion [mM]
ALDH-Bov	+	20	84.2 ± 1.4	16.8 ± 0.3
	+	40	35.1 ± 0.5	14.0 ± 0.2
	+	60	19.3 ± 0.2	11.6 ± 0.1
	+	80	11.3 ± 0.0	9.1 ± 0.0
	+	100	7.0 ± 0.0	7.0 ± 0.0
ALDH-Ec	+	20	86.7 ± 0.7	17.3 ± 0.1
	+	40	35.8 ± 0.1	14.3 ± 0.0
	+	60	19.1 ± 0.0	11.4 ± 0.0
	+	80	11.3 ± 0.2	9.0 ± 0.2
	+	100	7.0 ± 0.2	7.0 ± 0.2
empty *E. coli*	+	20	46.0 ± 0.9	9.2 ± 0.2
	+	40	6.0 ± 0.1	2.4 ± 0.1
	+	60	0.9 ± 0.0	0.5 ± 0.0
	+	80	0.3 ± 0.0	0.3 ± 0.0
	+	100	0.2 ± 0.0	0.2 ± 0.0

Table S15. Conversion [% and mM] to **51b** using lyophilized cells and increased substrate concentration without addition of NOx and NAD^+.

enzyme	NOx, NAD^+	substrate [mM]	conversion [%]	conversion [mM]
ALDH-Bov	-	20	87.6 ± 1.9	17.5 ± 0.4
	-	40	17.5 ± 0.6	7.0 ± 0.2
	-	60	3.2 ± 0.0	1.9 ± 0.0
	-	80	1.1 ± 0.1	0.9 ± 0.1
	-	100	0.5 ± 0.1	0.5 ± 0.1
ALDH-Ec	-	20	86.3 ± 1.0	17.3 ± 0.2
	-	40	5.4 ± 7.8	10.2 ± 3.1
	-	60	3.1 ± 0.0	1.9 ± 0.0
	-	80	1.2 ± 0.1	1.0 ± 0.0
	-	100	0.5 ± 0.1	0.5 ± 0.1
empty *E. coli*	-	20	43.6 ± 0.9	8.7 ± 0.2
	-	40	3.3 ± 0.2	1.3 ± 0.1
	-	60	0.6 ± 0.0	0.3 ± 0.0
	-	80	0.2 ± 0.0	0.2 ± 0.0
	-	100	0.1 ± 0.0	0.1 ± 0.0

12. Use of resting *E. coli* cells for biocatalytic reactions (51a and 54a)

The ALDH pellets (Bov and Ec) obtained from protein expression were resuspended in KPi buffer (50 mM, pH 8.5). Additionally *E. coli* BL21 DE3 cells containing empty pET28b plasmid were also tested.

Experimental conditions: Final volume 1 mL in Eppendorf tubes; resting cells (50 mg/mL) were rehydrated in KPi buffer (50 mM pH 8.5) containing DTT (100 µg/mL). In some reactions, NOx (5 µM, in purified form) and/or NAD^+ (0.5 mM) were added. The reactions were started by the addition of substrate **51a** or **54a** (20 mM, added as 1 M stock solutions in DMSO) and incubated on an orbital shaker at 170 rpm for 24 h at 40°C. The reactions were stopped by the acidic extraction with EtOAc, samples derivatized and conversions measured by GC-FID. Reactions were performed in duplicate.

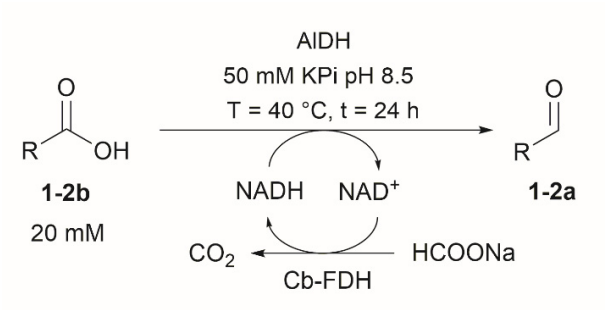
Table S16. Conversion [%] to **51b** using resting *E. coli* cells.

enzyme	NOx	NAD^+	DTT	conversion [%]
ALDH-Bov	+	+	+	67.8 ± 0.8
	+	-	+	67.4 ± 0.1
	-	+	+	67.4 ± 3.1
	-	-	+	68.3 ± 1.6
	-	-	-	67.2 ± 0.3
ALDH-Ec	+	+	+	70.9 ± 1.2
	+	-	+	73.3 ± 1.1
	-	+	+	70.5 ± 0.9
	-	-	+	70.4 ± 0.6
	-	-	-	70.3 ± 0.7
empty *E. coli*	+	+	+	32.6 ± 0.4
	+	-	+	32.3 ± 1.0
	-	+	+	30.5 ± 0.2
	-	-	+	31.3 ± 0.9
	-	-	-	31.3 ± 0.6

Table S17. Conversion [%] to **54b** using resting *E. coli* cells.

enzyme	NOx	NAD^+	DTT	conversion [%]
ALDH-Bov	+	+	+	63.6 ± 5.0
	+	-	+	15.3 ± 0.1
	-	+	+	64.0 ± 2.1
	-	-	+	16.9 ± 0.1
	-	-	-	15.3 ± 0.2
ALDH-Ec	+	+	+	95.5 ± 0.5
	+	-	+	91.8 ± 2.1
	-	+	+	93.5 ± 0.3
	-	-	+	88.9 ± 0.1
	-	-	-	90.0 ± 0.1
empty *E. coli*	+	+	+	6.1 ± 0.1
	+	-	+	4.7 ± 0.1
	-	+	+	5.9 ± 0.2
	-	-	+	4.5 ± 0.3
	-	-	-	4.5 ± 0.3

13. Testing for the reverse reaction: reduction of carboxylic acid to aldehyde



Experimental conditions: 1 mL final volume in Eppendorf tubes, buffer = 50 mM KPi pH 8.5, 180 rpm on orbital shaker, T = 40 °C, [**1b**] or [**2b**] = 20 mM, [NAD^+] = 1 mM, [ALDH] = 10 µM, [Cb-FDH] = 10 µM, [HCOONa] = 0.5 M, [DTT] = 100 µg/mL.

After 24 h reaction time, the reactions were split. 400 µL were extracted under basic conditions using toluene as IS and the formation of the aldehyde was analyzed by GC-FID (DB-1701). 400 µL of the reaction were extracted under acidic conditions using toluene as IS and the acid was derivatized to the methylester with (trimethylsilyl)diazomethane. Conversions were measured by GC-FID (DB-1701).

The FDH from *Candida boidinii* (Cb-FDH) was expressed and purified as described previously. [4]

Table S18. Results for the formation of aldehyde from acid [%].

substrate	ALDH	aldehyde [%]	recovered acid [%]
1b	ALDH-Bov	n.d.	93 ± 6
1b	ALDH-EC	n.d.	94 ± 6
1b	PP-ALDH	n.d.	96 ± 10
1b	Blank [a]	n.d	93 ± 1
2b	ALDH-Bov	n.d.	97 ± 1
2b	ALDH-EC	n.d.	96 ± 6
2b	PP-ALDH	n.d.	93 ± 6
2b	Blank [a]	n.d.	94 ± 5

[a] The Blank reaction did contain everything, except ALDH.

14. Thermostability measurements

The thermostability (melting temperature T_m) of the three ALDHs and NOx was determined by differential scanning fluorometry using a Biorad-7500 QPCR system. The T_m, defined as the temperature at which 50% of the enzyme unfolds, was determined for each enzyme using different buffers (50 mM Tris-HCl pH 7, 50 mM Tris-HCl pH 8, 50 mM Tris-HCl pH 8.5, 50 mM Tris-HCl pH 9, 50 mM KPi pH 8, 50 mM KPi pH 8.5, 50 mM KPi pH 9). Each reaction mixture (20 µL) contained of SYPRO orange (5000x) as fluorescence dye in the final concentration of 1x (20x stock in qH_2O), 2 µg of enzyme and the buffer. For the ALDHs, also DTT (100 µM) and NAD^+ (50 µM) were added.

Fluorescence data were collected as a continuous standard melt curve from 20- 90 °C (1% increment, hold 1 min at 20 °C and 1 min at 90 °C), using ROX for reporter and none for quencher. Also none was selected as passive reference. Two reaction replicates of each enzyme were prepared for every buffer tested.

Table S19. Melting temperature T_m [°C] for the enzymes used in this study.

	ALDH-Bov		ALDH-EC		PP-ALDH		NOx
DTT and NAD^+	**-**	**+**	**-**	**+**	**-**	**+**	**-**
50 mM Tris/HCl pH 8	48	50	48	50	37	37	52
50 mM Tris/HCl pH 8.5	48	50	48	50	39	39	52
50 mM Tris/HCl pH 9	46	49	49	48	38	40	50
50 mM KPi pH 8	45	48	44	46	43	42	51
50 mM KPi pH 8.5	44	47	38	44	42	42	51
50 mM Gly/NaOH pH 9	47	48	42	47	41	42	53

Note: Purified ALDHs as well as lyophilized whole cells containing overexpressed ALDHs did not lose activity when stored at -80 °C for more than 2 years.

15. Analytical methods

The conversions were measured by GC-FID using an Agilent J&W DB1701 (30 m, 250 µm, 0.25 µm) column. For substrate **9**, an Agilent HP5 column (30 m, 320 µm, 0.25 µm) was used. Carrier gas: H_2

Method A (HP5): constant pressure 4 psi; temperature program: 60 °C, hold 20 min; 20 °C min^{-1} to 280 °C, hold 5 min.

Method B (DB1701): constant pressure 6.9 psi; temperature program: 60 °C, hold 6.5 min; 20 °C min^{-1} to 100 °C, hold 1 min; 20 °C min^{-1} to 280 °C, hold 1 min.

Method C (DB1701): constant pressure 6.9 psi; temperature program: 60 °C, hold 4.5 min; 20 °C min^{-1} to 80 °C, hold 1 min; 20 °C min^{-1} to 280 °C, hold 5 min.

Method D (DB1701): constant pressure 6.9 psi; temperature program: 60 °C, hold 4.5 min; 20 °C min^{-1} to 80 °C, hold 1 min; 20 °C min^{-1} to 280 °C, hold 10 min.

Table S20. Retention times [min].

number	aldehyde	acid[a]	method	number	aldehyde	acid[a]	method	number	aldehyde	acid[a]	method
1	4.3	6.8	B	**21**	9.3	11.6	C	**41**	14.7	15.2	C
2	10.4	12.3	B	**22**	9.5	10.9	C	**42**	14.3	15.0	C
3	[b]	3.4	B	**23**	9.6	10.9	C	**43**	14.6	15.2	C
4	3.3	5.5	B	**24**	9.9	10.8	C	**44**	14.6	15.2	C
5	3.3	5.2	B	**25**	11.7	13.2	C	**45**	13.6+14.8+15.2	15.0+15.3+15.4	C
6	6.7	9.3	B	**26**	11.9	13.0	C	**46**	13.9	14.2	C
7	5.3	8.6	B	**27**	12.0	12.9	C	**47**	9.0	10.3	C
8	7.7	9.7	A	**28**	13.3	13.6	C	**48**	13.1	13.8	C
9	8.3	10.6	B	**29**	12.8	14.0	C	**49**	11.6+12.3	13.9	C
10	9.2	10.5	C	**30**	12.9	13.8	C	**50**	7.9	10.8	B
11	10.5	11.5	C	**31**	12.9	13.8	C	**51**	13.6	14.1	C
12	11.6	12.4	C	**32**	13.4	13.9	C	**52**	11.6	12.7	B
13	9.4	11.4	B	**33**	13.2	13.8	C	**53**	11.4	12.1	C
14	11.8	12.5	C	**34**	10.2+12.7	11.8	C	**54**	13.3	13.9	B
15	13.0	13.5	C	**35**	12.2+14.4	13.2+13.7+14.9	C	**55**	13.4	14.3	C
16	17.0+17.1	17.2+17.3+17.4	C	**36**	13.0+15.2	13.7+14.3+15.4	C	**56**	15.1	15.6	C
17	11.2	12.4	C	**37**	12.7	13.3	C	**57**	14.8	15.5	C
18	11.2	12.4	C	**38**	12.2	13.2	C	**58**	14.9	15.6	C
19	11.4	12.5	C	**39**	12.9	13.6	C	**59**	17.5	17.8	D
20	12.8	13.0	C	**40**	15.2	15.4	C	**60**	14.8	15.4	C

[a] measured as derivatized ester

[b] elutes with the solvent

Note: multiple peaks occured due to a partial methylation of the hydroxyl group. Also the silyl-intermediate could be detected in some cases.

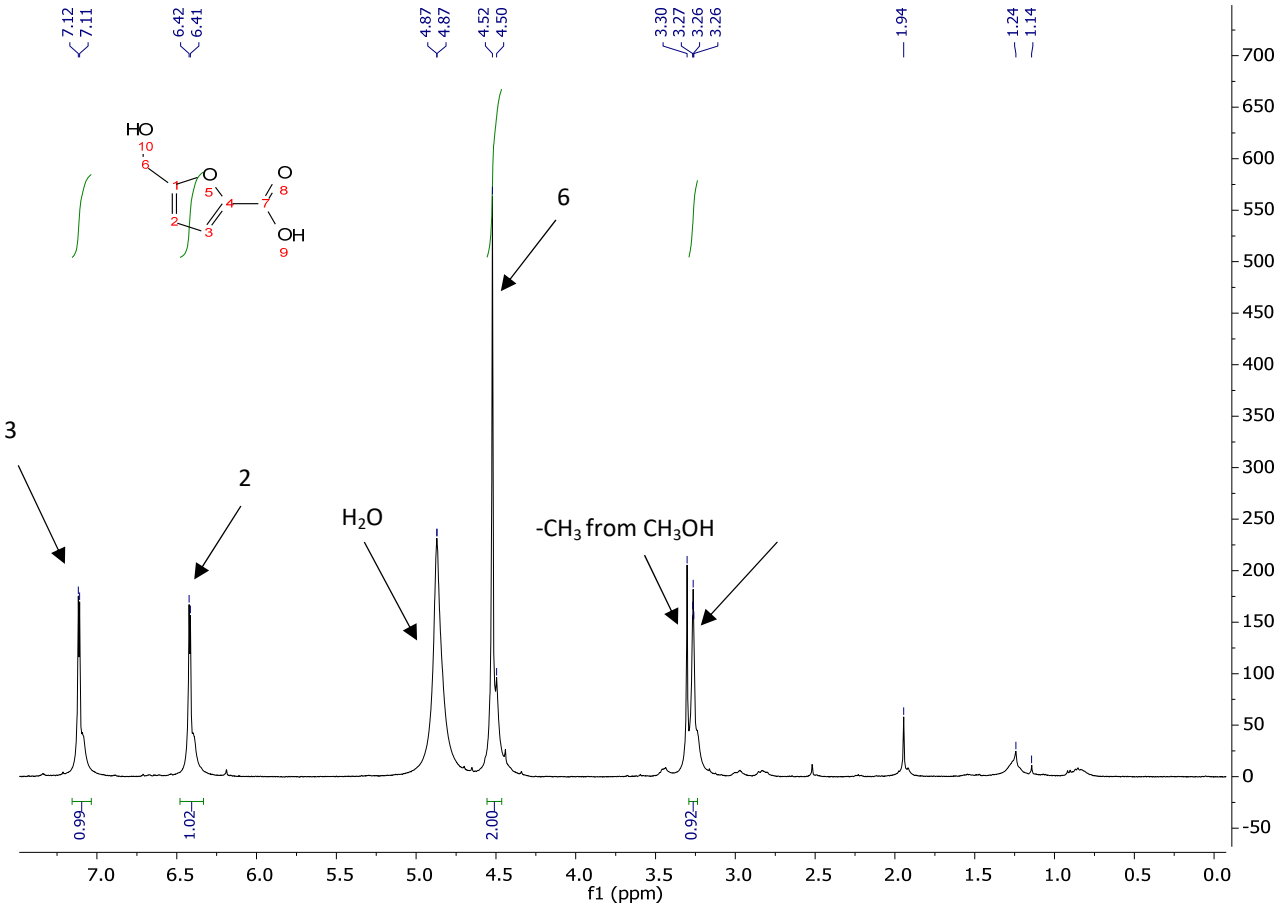
The enantiomeric excess for the aldehyde **1a** was measured on a Restek Rt-ßDEXsa column (30m, 0.25 mm, 0.25 µm); split ratio 10:1; injector 180 °C, detector 200 °C, flow 0.9 mL/min; Temp. program: 80 °C hold 6.5 min, 10 °C/min to 230 °C hold 1 min. Retention times: (*R*)-**1a** 10.22 min, (*S*)-**1a** 10.37 min. The absolute configuration was assigned by comparison with literature data.[1]

The enantiomeric excess for **1b** and **61b** (as methyl ester) were measured on a Restek Rt-ßDEXsm column (30m, 0.25 mm, 0.25 µm); split ratio 10:1;, injector 180 °C, detector 200 °C, flow 0.9 mL/min; Temp. program: 60 °C hold 6.5 min, 10 °C/min to 230 °C hold 1 min. Retention times: (*R*)-**1b**-methyl ester 10.61 min, (*S*)-**1b**-methyl ester 10.74 min. Retention times 61b: (*S*)-**61b** 17.33 min, (*R*)-**61b** 17.36 min. The absolute configuration was assigned by comparison with literature data.[1,5]

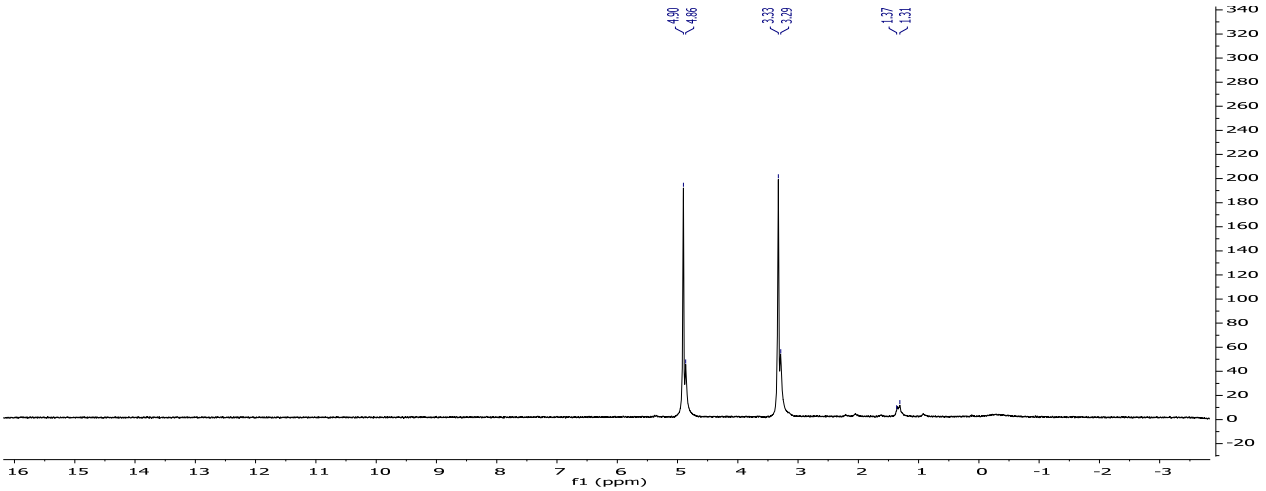
16. NMR spectra of upscaling

^{1}H NMR spectra were recorded on a Brucker (400 MHz) spectrometer in CD_3OD. All signals are expressed as ppm down field from tetramethylsilane.

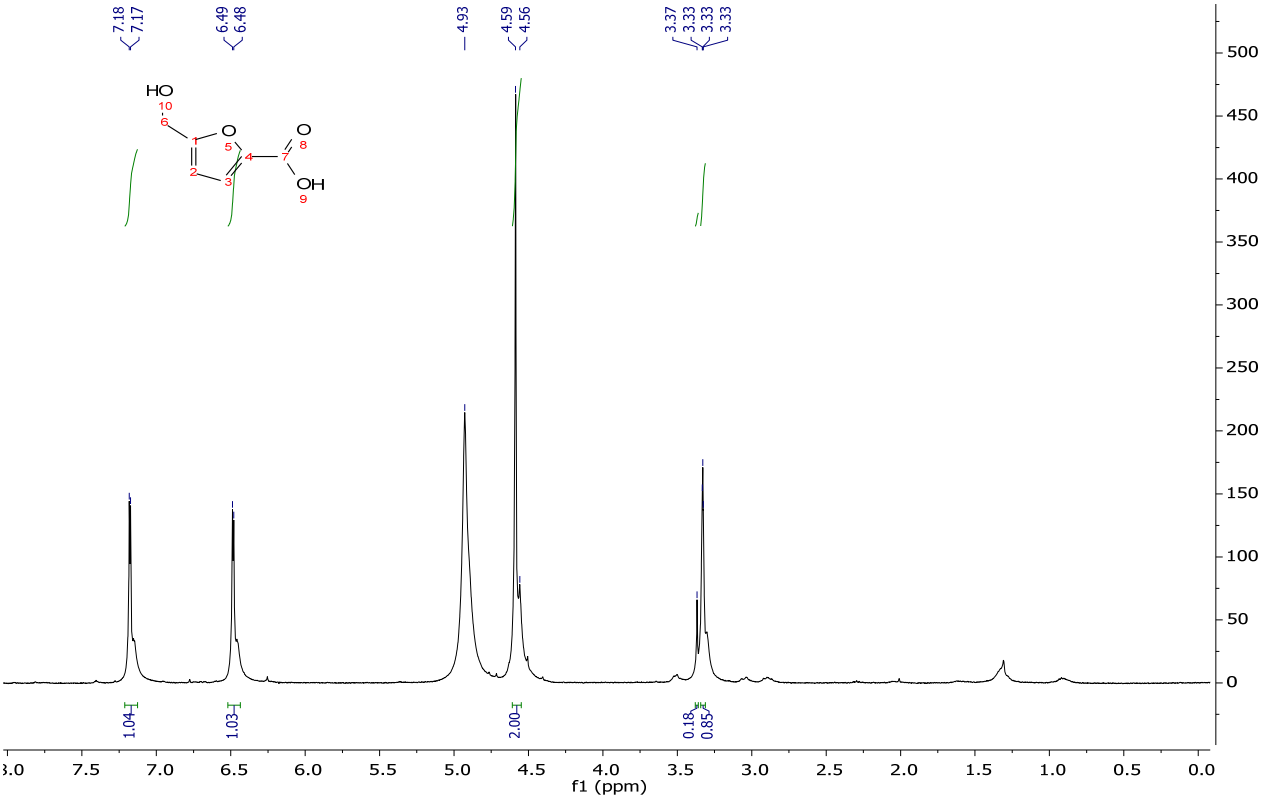
(a) ^{1}H-NMR spectrum upscaling 51a with ALDH-Bov (lyophilized cells) in CD_3OD.



(b) ^{1}H-NMR spectrum of pure CD_3OD.



(c) ^{1}H-NMR spectrum upscaling 51a with ALDH-Ec (lyophilized cells) in CD_3OD.



17. References

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