Supporting Information

Highly Efficient and Scalable Chemoenzymatic Syntheses of (R)- and (S)-Lactaldehydes

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1. Screening of ketoreductases

sample	enzyme no. from kit	rel. peak area: starting ketone	rel. peak area: sum of enantiomeric hydroxy compounds	enantiomeric ratio: 1st : 2nd eluting enantiomer
T23247-001	1	0%	100%	67.6 : 32.4
T23247-002	2	0%	100%	12.6 : 87.4
T23247-003	3	0%	100%	0.5 : 99.5
T23247-004	4	0%	100%	0.0 : 100.0
T23247-005	5	0%	100%	99.0 : 1.0
T23247-006	6	0%	100%	100.0 : 0.0
T23247-007	7	0%	100%	98.8 : 1.2
T23247-008	8	10.2%	89.8%	86.9 : 13.1
T23247-009	9	0%	100%	100.0 : 0.0
T23247-010	10	0%	100%	100.0 : 0.0
T23247-011	11	0%	100%	70.1 : 29.9
T23247-012	12	0%	100%	71.3 : 28.7
T23247-013	13	0%	100%	99.5 : 0.5
T23247-014	14	0%	100%	51.8 : 48.2
T23247-015	15	0%	100%	69.1 : 30.9
T23247-016	16	0%	100%	100.0 : 0.0
T23247-017	17	0%	100%	84.7 : 15.3
T23247-018	18	0%	100%	94.6 : 5.4
T23247-019	19	0%	100%	95.4 : 4.6
T23247-020	20	0%	100%	100.0 : 0.0
T23247-021	21	0%	100%	100.0 : 0.0
T23247-022	22	0%	100%	0.0 : 100.0
T23247-023	23	0%	100%	72.9 : 27.1
T23247-024	24	0%	100%	0.8 : 99.2
T23247-025: starting material (ref.)		100%	0%	-
T23247-026: racemic product (ref.)		0%	100%	50.2 : 49.8

Enzyme screening: Starting conditions of the screening were given by a loading of substrate 3 of 6 g/L, KRED loading of 1 g/L and a NADPH loading of 0.8 g/L in potassium phosphate buffer of pH=7. The screening reactions were conducted in 5mL glass vials, placed in heating blocks of a magnetic stirrer at 30°C. The reaction course was monitored by TLC (silicagel, AcOEt:Heptane=1:1, Phosphomolybdic acid solution, heat to 120°, starting material hardly visible) and conversion and enantiomeric ratio were determined by GC (SupelcoWax 10 capillary GC column 30m x 0.25mm and β-Dex 110 column 30m x 0.25mm respectively) (see section 3 of the supporting information)

3. Scale-up of ketoreductase-catalyzed reduction of Methylglyoxal-1,1-dimethylacetal

Preparation of 0.1M KH_2PO_4-buffer solution (pH = 6.9-7.1): 473mL of 0.25M K_2HPO_4-solution (prepared from 34.85g (0.2 mol) K_2HPO_4 and 800mL deionized water, pH = 9.25) and 296mL of 0.25M KH_2PO_4-solution (prepared from 17.01g (0.12mol) KH_2PO_4, 305mg (2.53mmol) magnesium sulfate and 500mL deionized water, pH = 4.4) were added to 1154mL of deionized water at 20-30°C.

Preparation of (R)-1,1-Dimethoxy-2-propanol: 1960g 0.1M KH_2PO_4-buffer solution was placed in a 6.0L reaction flask, equipped with overhead stirring. 474.6g (4.018 mol) Methylglyoxal-1,1-dimethylacetal dissolved in 348g IPA, and 261mg (0.341 mol) NADP+ dissolved in 22g 0.1M KH_2PO_4-buffer solution were subsequently added to the flask. A solution of 3.01g KRED-P2-G03 in 150g 0.1M KH_2PO_4-buffer solution was added to start the reaction, applying gentle stirring at ≤100 rpm. With completed addition the reaction mixture was heated to 30 °C and stirred for 24 h. To the turbid solution 750g MTBE were added. The organic layer was saturated with 700 g sodium chloride and stirred for 30 min at 20-30°C. The reaction mixture was transferred into a separating funnel and further 3.5kg MTBE were added. After extraction, the layers were separated and the aqueous layer further extracted with 3 x 4kg MTBE. The combined organic layers were dried over 400g $MgSO_4$, the solid filtered off and washed two times with 100g MTBE. The solvents were evaporated carefully under reduced pressure. The crude product was distilled using a 30 cm Vigreux column at 70°C and 85 mbar to give 393.1g (3.27 mol, 81.4%) (R)-1,1-dimethoxy-2-propanol as a colorless liquid.
Analytical data: GC: 99.5%, ee: 99.9% (measured by chiral stationary phase GC), ^{1}H-NMR (d^6-DMSO): δ 4.55 (bs, 1H), 3.98 (d, J = 5.8 Hz, 1H), 3.56 (dq, J = 6.4, 5.8 Hz, 1H), 3.30 (s, 6H), 1.00 (d, J = 6.4 Hz, 3H).

3. GC – Chemical purity of (S)-1,1-Dimethoxy-2-propanol

768936 (S)-1,1-Dimethoxy-2-propanol

Lot Number: **BCBN5894**	Sample Name: **00000000481198_001_GC**

Agilent 6890N (536GC34)

Injector:	250 °C	***GC Serial Number:***	CN10628006
Detector (FID):	280 °C	***Injection Time:***	11.12.14 18:39
Carriergas:	Helium	***Sample Type:***	unknown
Column flow:	1.8 ml/min	***Channel:***	GC_1
Temp. Control:	see Figure 1	***Sequence Creation Time:***	01.12.14 15:22
Split Ratio:	100:1	***Sequence Created By:***	Patrick Stocker
Range:	0	***Time Processed:***	15.12.14 15:28
Injection Volume:	1.0 ul	***Processed By:***	Patrick Stocker
Vial Number:	13		
Run Time:	20.00 min		
Column:	Supelcowax 10 (30m x 0.25mm, 0.50um)		
Sample Prep.:	50 ul in 1 ml THF		

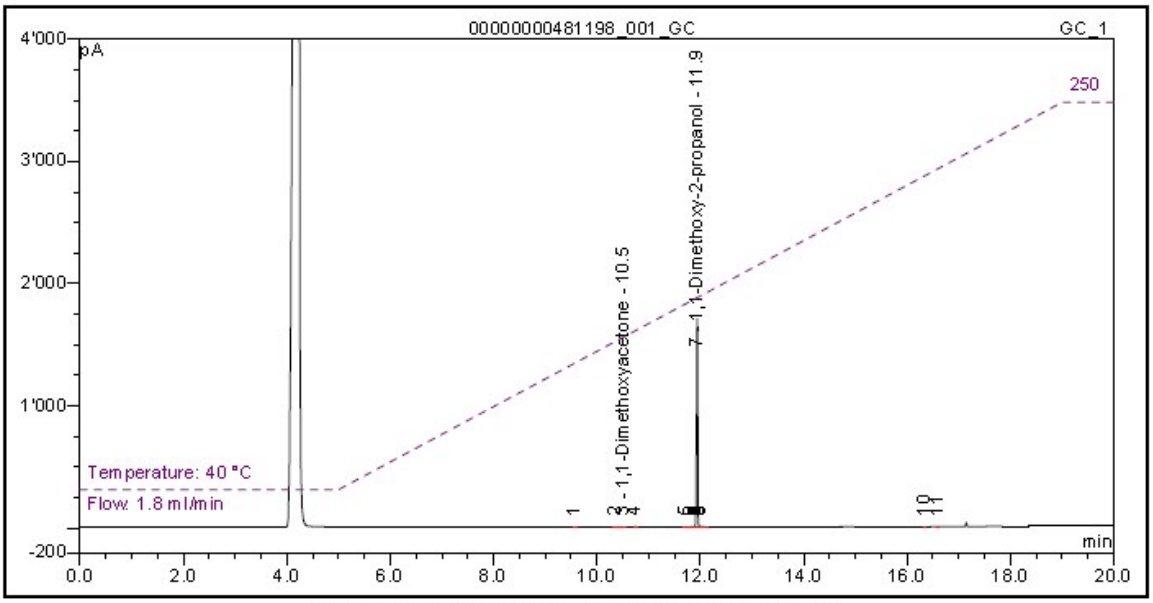
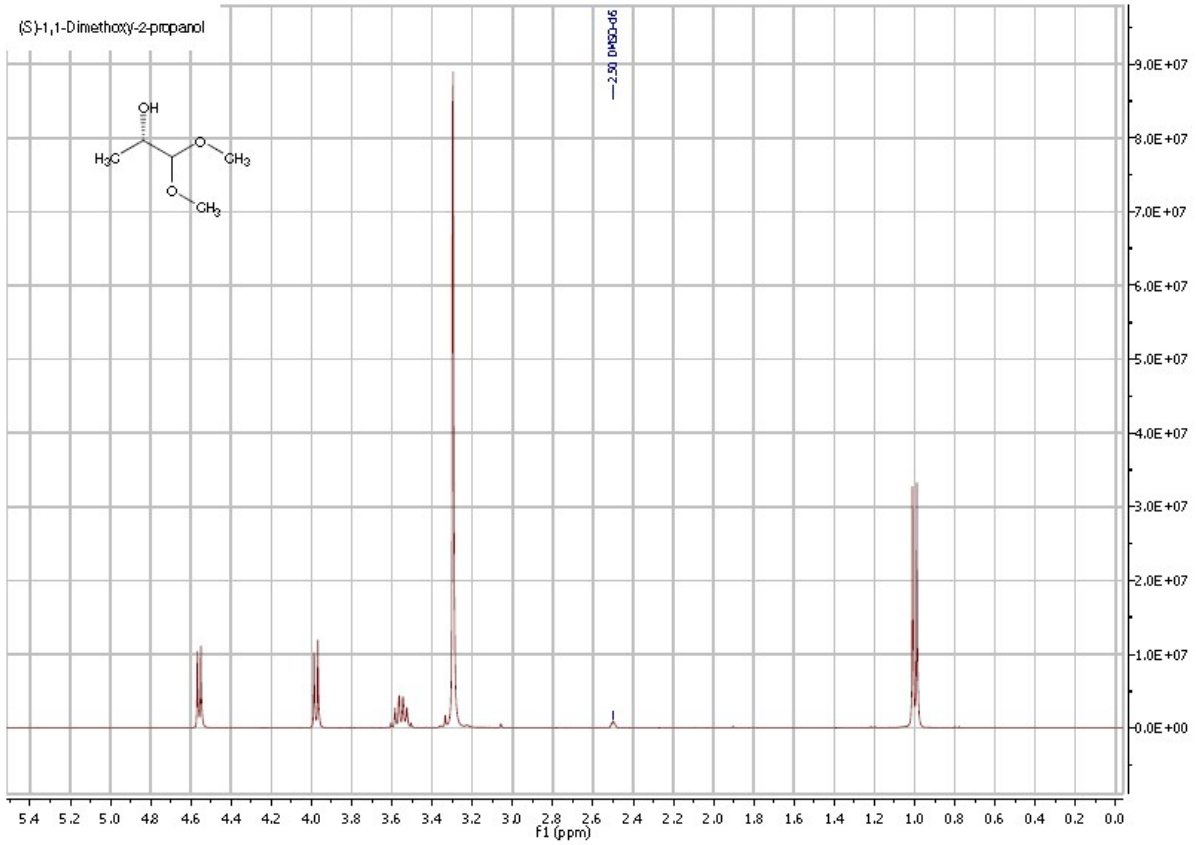


Figure 1: Zoomed Chromatogram and Temperatur Program

No.	Ret.Time min	Peak Name	Type	Height pA	Area pA*min	Amount w/w%	Rel.Area %
1	9.580	n.a.	BMB	0.8150	0.0236	n.a.	0.05
2	10.368	n.a.	BMB*	0.2926	0.0086	n.a.	0.02
3	10.511	1,1-Dimethoxya	BMB	1.1917	0.0306	n.a.	0.07
4	10.745	n.a.	BMB	0.9974	0.0264	n.a.	0.06
5	11.730	n.a.	BMB*	0.3021	0.0086	n.a.	0.02
6	11.829	n.a.	BMB*	0.5230	0.0132	n.a.	0.03
7	11.941	1,1-Dimethoxy-2	BM *	1703.0924	45.6646	n.a.	99.49
8	11.981	n.a.	M *	4.0453	0.0769	n.a.	0.17
9	12.015	n.a.	MB*	0.7793	0.0291	n.a.	0.06
10	16.339	n.a.	BMB*	0.4749	0.0102	n.a.	0.02
11	16.595	n.a.	BMB*	0.4310	0.0091	n.a.	0.02
Total:				1712.9447	45.9009		100.000

4. NMR of (S)-1,1-Dimethoxy-2-propanol



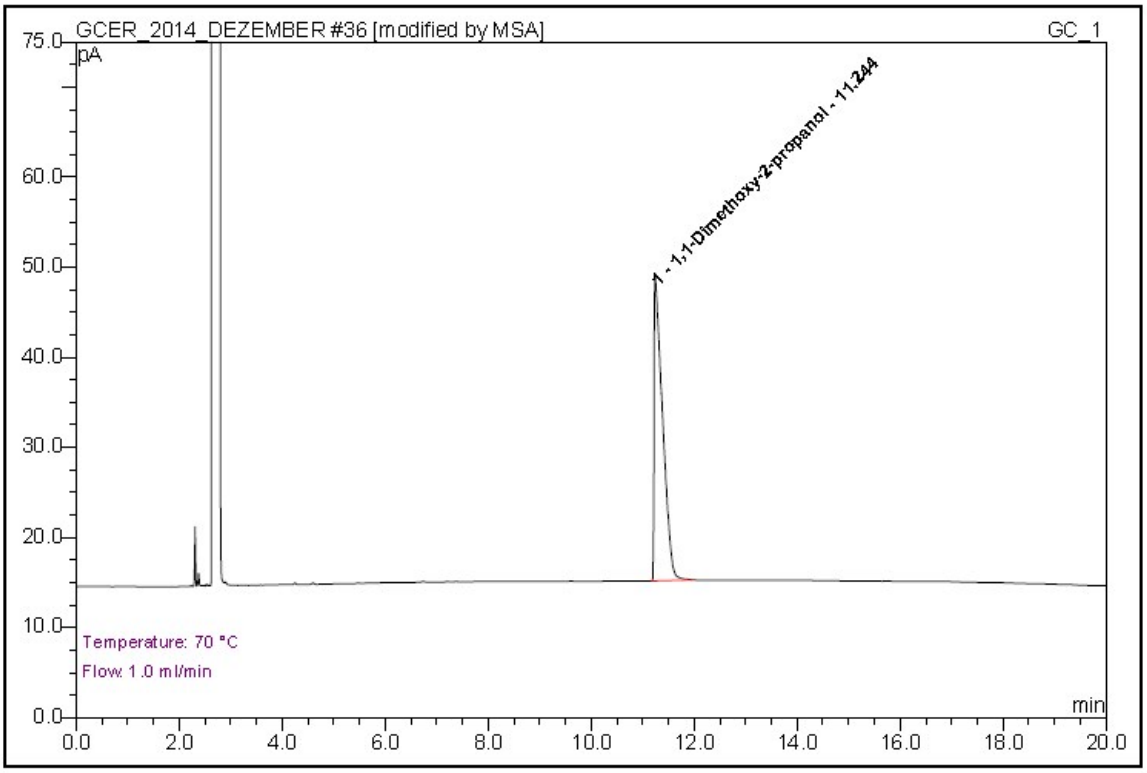
5. Enantiomeric purity analysis of (S)- and (R)-1,1-Dimethoxy-2-propanol by GC

768936 (S)-1,1-Dimethoxy-2-propanol

Lot	**BCBN5894**	***Sample:***	000000000481198_001_ER

Injector (SSI, °C)	250	*Channel:*	GC_1
Detector (FID, °C)	250	*Vial Number:*	23
Carriergas:	Helium	*Injection Volume [µl]:*	1.0
Column Flow (ml/min)	1.0	*Range:*	-
Split ratio:	100:1	*Run Time (min):*	20.00
Quantif. Method:	768936	*Recording Time:*	15.12.14 07:53
Column:	Supelco beta-DEX 110 30 m x 0.25 mm 0.25 µm		

Sample Preparation 10 ul in 1.5 ml TBME



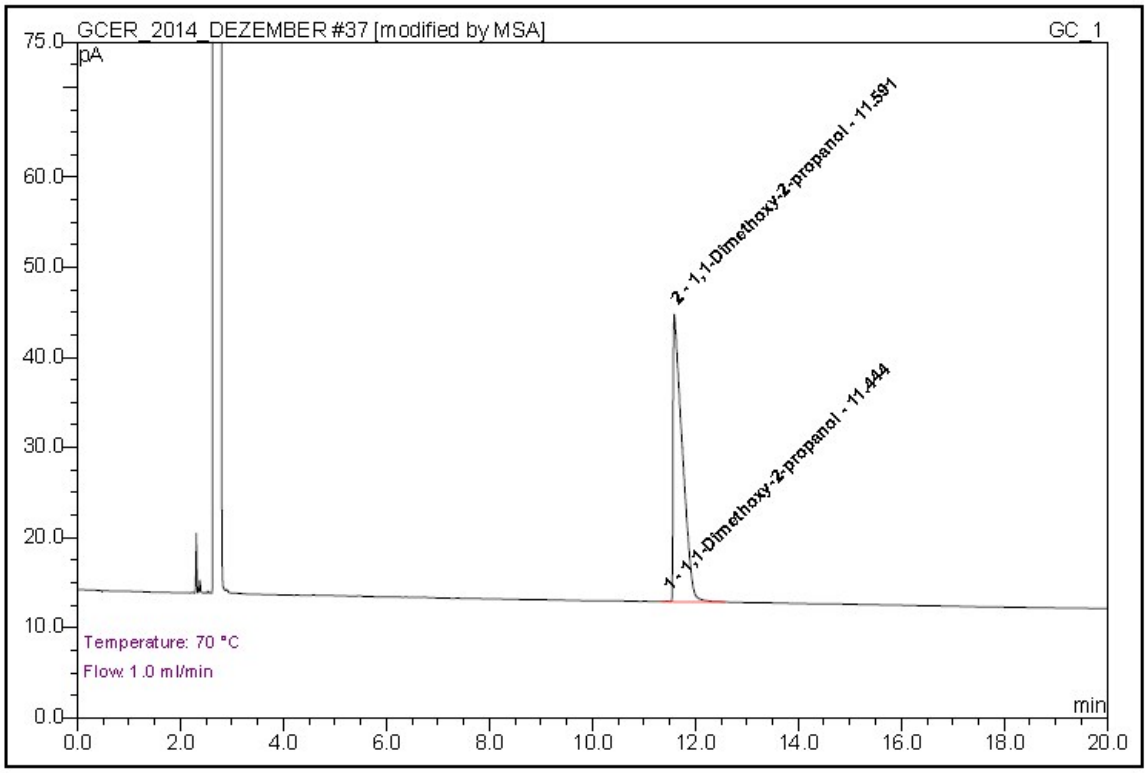
No.	Ret.Time min	Peak Name	Height pA	Area pA*min	Type	Amount	Rel.Area %
1	11.24	1,1-Dimethoxy-	34.107	6.4481	BMB*	n.a.	100.000
Total:			34.107	6.4481	0.00	0.000	100.000

768928 (R)-1,1-Dimethoxy-2-propanol

Lot **BCBM8110** ***Sample:*** 000000000494930_001_ER

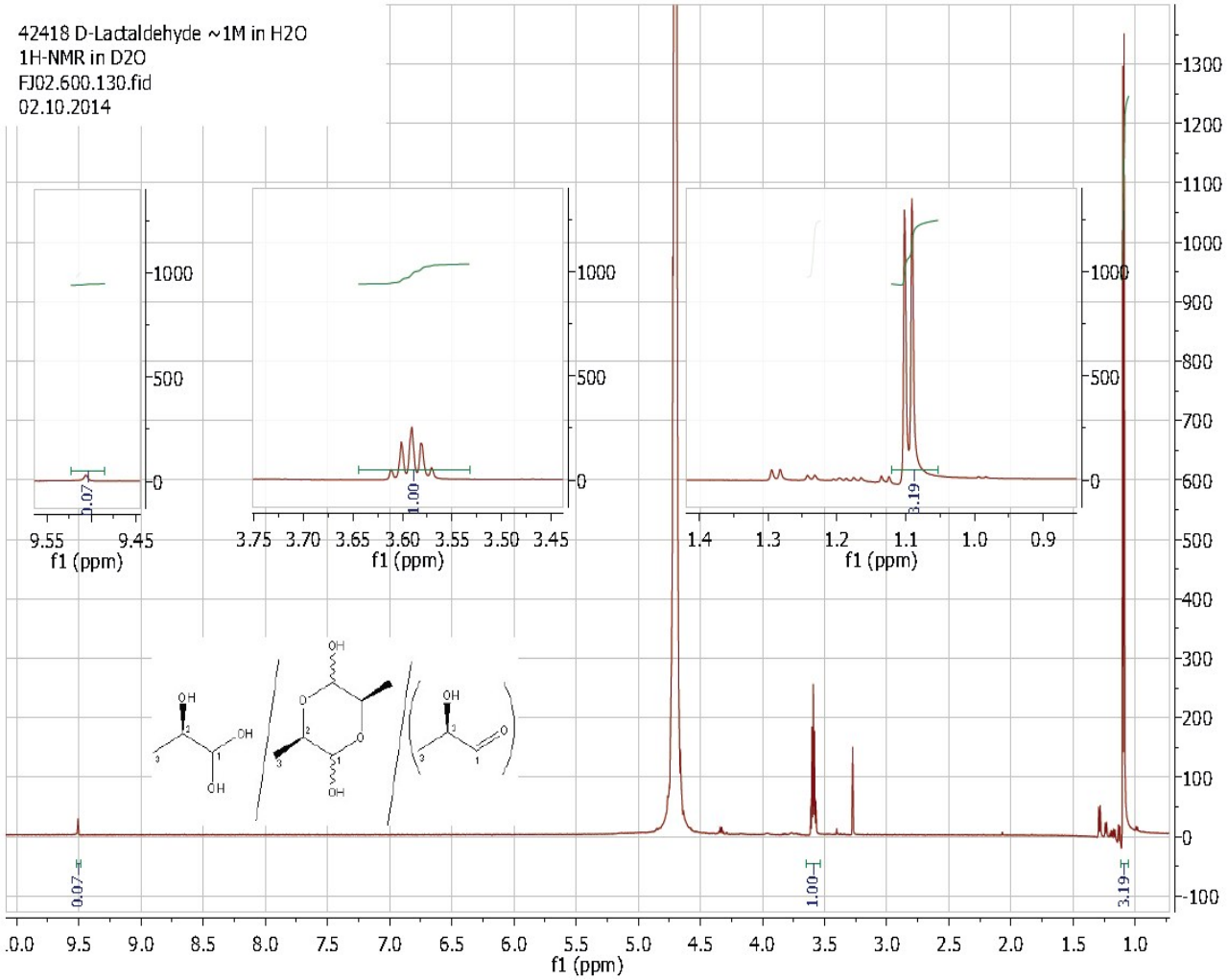
Injector (SSI, °C)	250	*Channel:*	GC_1
Detector (FID, °C)	250	*Vial Number:*	24
Carriergas:	Helium	*Injection Volume [µl]:*	1.0
Column Flow (ml/min)	1.0	*Range:*	-
Split ratio:	100:1	*Run Time (min):*	20.00
Quantif. Method:	768928	*Recording Time:*	15.12.14 08:15
Column:	Supelco beta-DEX 110 30 m x 0.25 mm 0.25 µm		

Sample Preparation 10 ul in 1.5 ml TBME

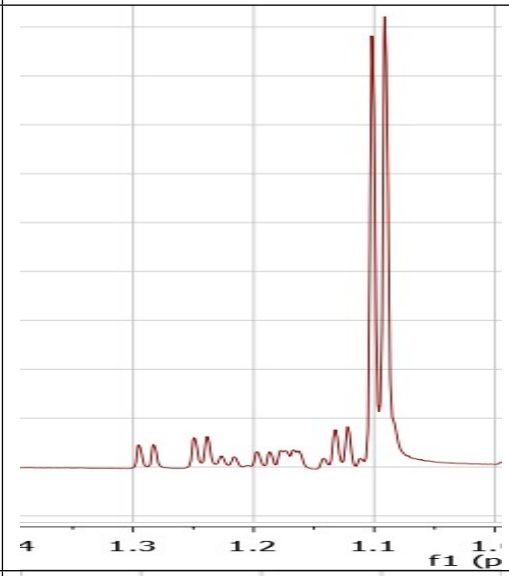
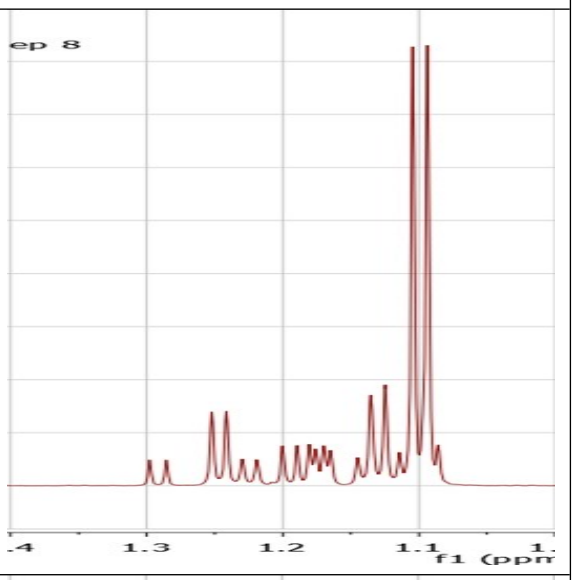
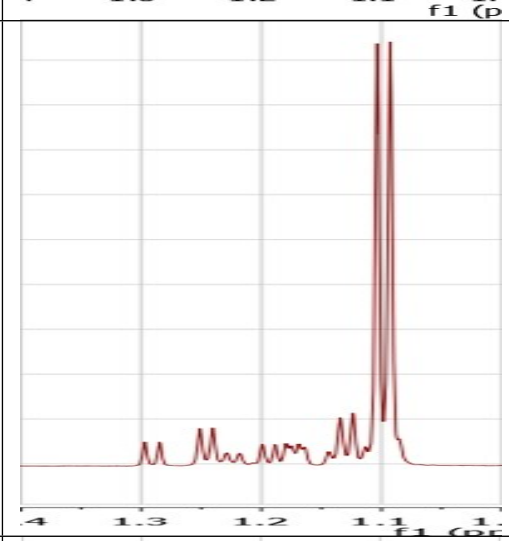
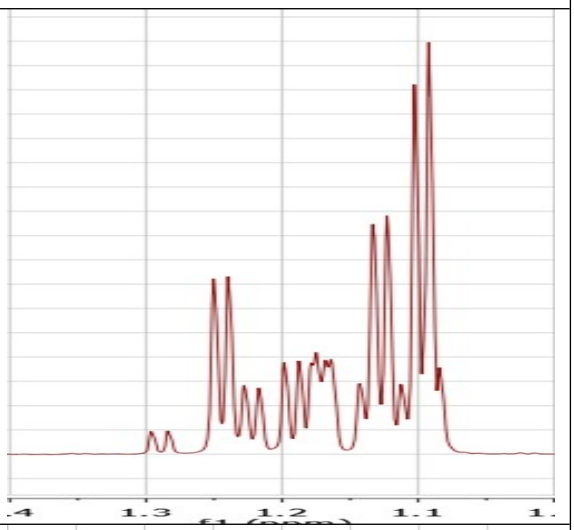
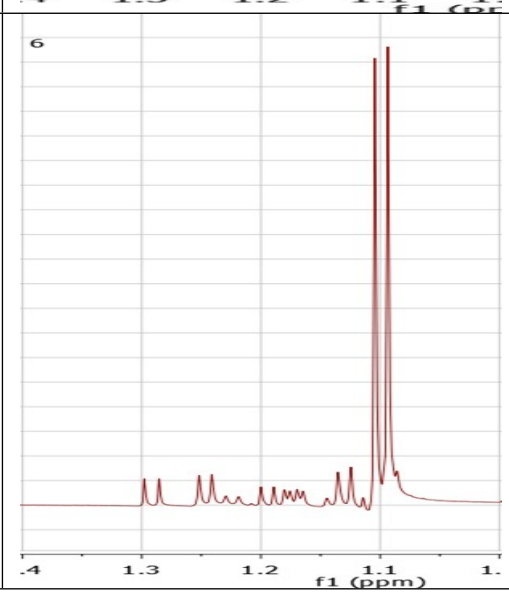
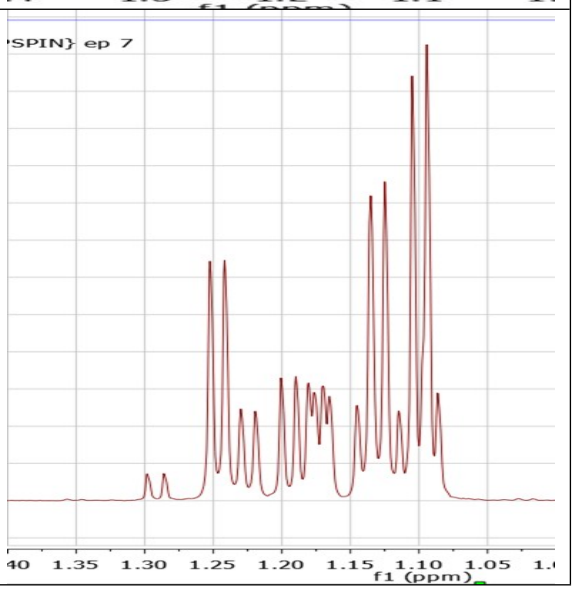


No.	Ret.Time min	Peak Name	Height pA	Area pA*min	Type	Amount	Rel.Area %
1	11.44	1,1-Dimethoxy	0.053	0.0043	BMb*	n.a.	0.066
2	11.59	1,1-Dimethoxy	31.915	6.5334	bMB*	n.a.	99.934
Total:			31.968	6.5377	0.00	0.000	100.000

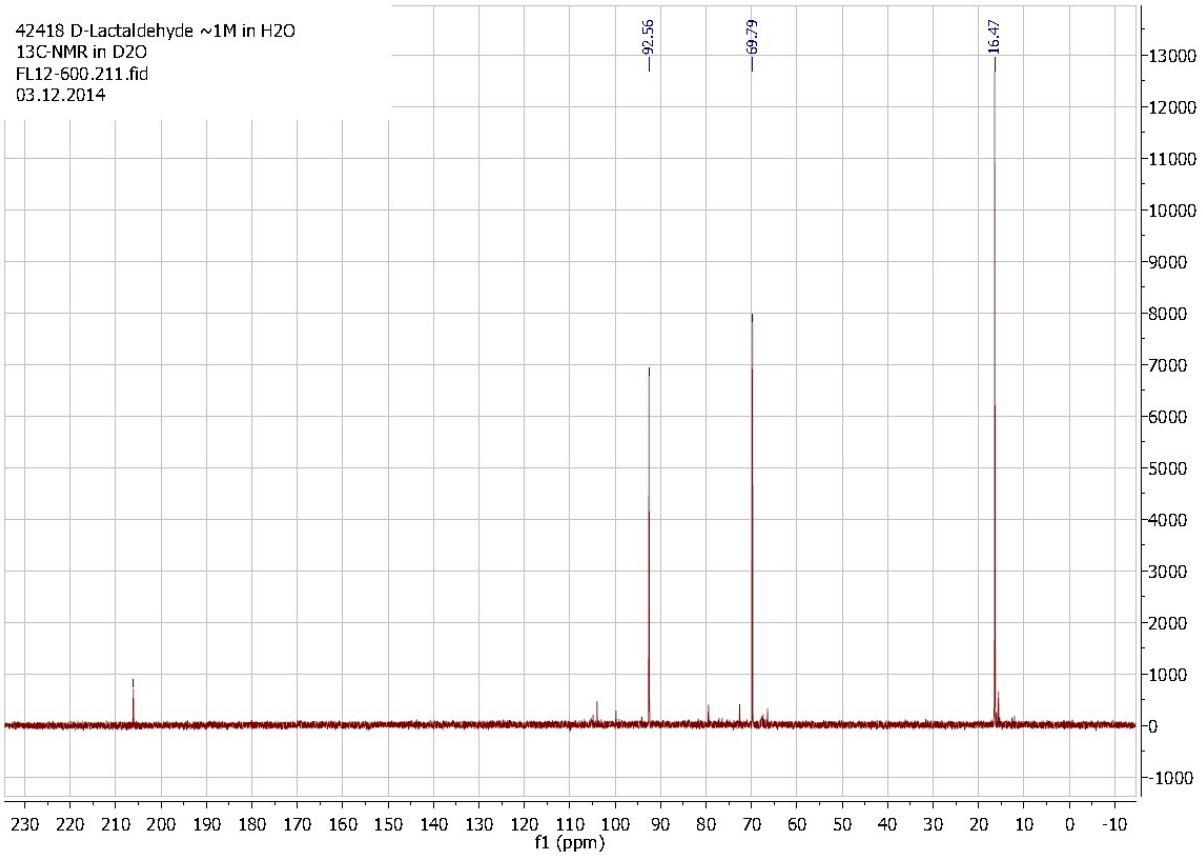
6. ^{1}H-NMR of (R)-Lactaldehyde



7. Stability of (R)-Lactaldehyde by ^{1}H-NMR, signal of methyl group

storage, r.t.	aqueous solution	neat compound
1 day		
4 days		
21 days		

8. ^{13}C-NMR of (R)-Lactaldehyde



9. Enantiomeric purity analysis of (S)- and (R)-lactaldehyde as its dinitrophenylhydrazone derivatives by HPLC

47014 L-Lactaldehyde solution (as its DNPH derivative)

Lot Number: **BA**	Sample Name: **T23584_002**

Dionex Summit (536LC25)

Pump :	P680	***Injection Time:***	03.11.14 15:55
Autosampler:	ASI-100	***Processed By:***	Hansjoerg Tinner
Detector:	UVD 340U	***Vial Number:***	GA4
Column:	Daicel Chiralcel OD, 5 um (S/N: n.a.)	***Sample Type:***	unknown
Column Dim.:	250 mm x 4.6 mm		
Mobile Phase:		***Injection Volume:***	10.0 µl
%A :	0 %	***Flow:***	1.00 ml/min
%B :	0 %	***Column Temp. (°C):***	25.0
%C :	2-Propanol 15 %	***Run Time:***	36.00 min
%D :	Hexan 85 %		
Gradient :	iso		
Sample Prep.:	2 mg in 10 ml 2-Propanol : Hexan 2 : 8		

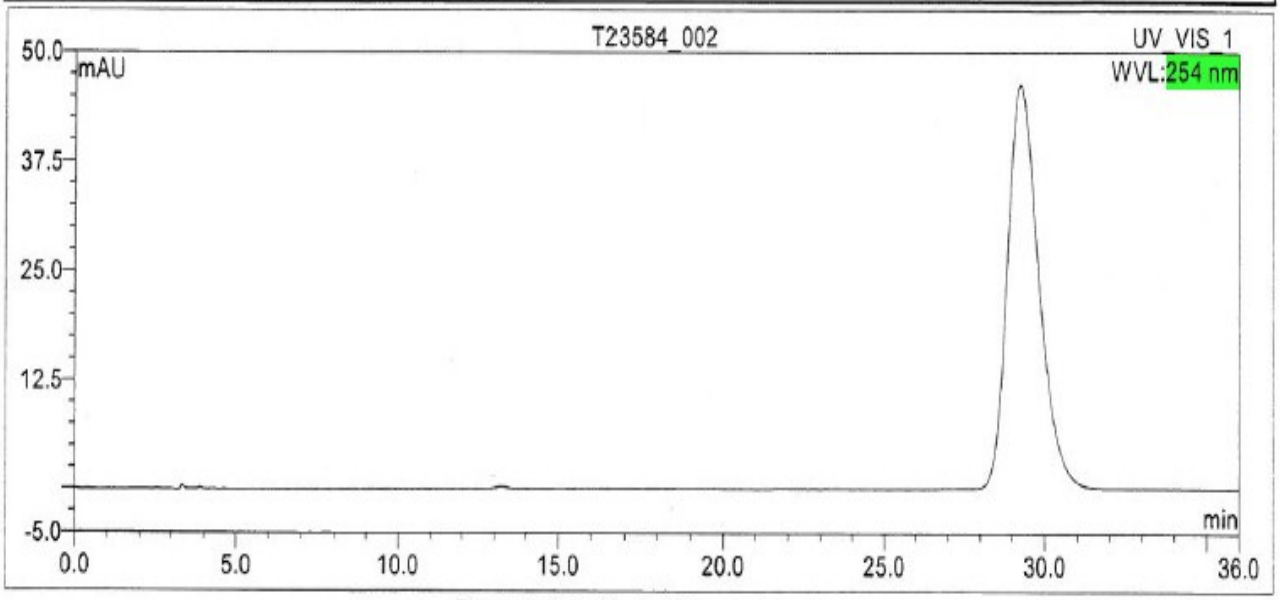


Figure 1: Auto-Scaled Chromatogram

42418 D-Lactaldehyde solution (as its DNPH derivative)

Lot Number: **BA**	Sample Name: **T23583_002**

Dionex Summit (536LC25)

Pump :	P680	***Injection Time:***	03.11.14 16:32
Autosampler:	ASI-100	***Processed By:***	Hansjoerg Tinner
Detector:	UVD 340U	***Vial Number:***	GA5
Column:	Daicel Chiralcel OD, 5 um (S/N: n.a.)	***Sample Type:***	unknown
Column Dim.:	250 mm x 4.6 mm		
Mobile Phase:		***Injection Volume:***	10.0 µl
%A :	0 %	***Flow:***	1.00 ml/min
%B :	0 %	***Column Temp. (°C):***	25.0
%C :	2-Propanol 15 %	***Run Time:***	38.00 min
%D :	Hexan 85 %		
Gradient :	iso		
Sample Prep.:	2 mg in 10 ml 2-Propanol : Hexan 2 : 8		

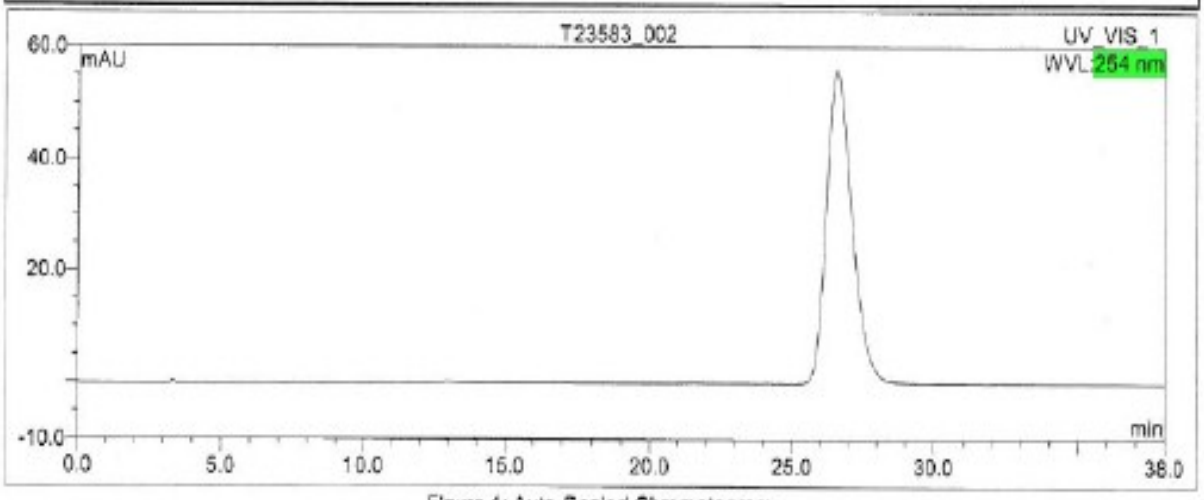


Figure 1: Auto-Scaled Chromatogram

49426 DL-Lactaldehyde solution (as its DNPH derivative)

Lot Number: **BA**	Sample Name:

Dionex Summit (536LC25)

Pump :	P680	***Injection Time:***	03.11.14 17:10
Autosampler:	ASI-100	***Processed By:***	Hansjoerg Tinner
Detector:	UVD 340U	***Vial Number:***	GA3
Column:	Daicel Chiralcel OD, 5 um (S/N: n.a.)	***Sample Type:***	unknown
Column Dim.:	250 mm x 4.6 mm		
Mobile Phase:		***Injection Volume:***	10.0 µl
%A :	0 %	***Flow:***	1.00 ml/min
%B :	0 %	***Column Temp. (°C):***	25.0
%C :	2-Propanol 15 %	***Run Time:***	38.00 min
%D :	Hexan 85 %		
Gradient :	iso		
Sample Prep.:	2 mg in 10 ml 2-Propanol : Hexan 2 : 8		

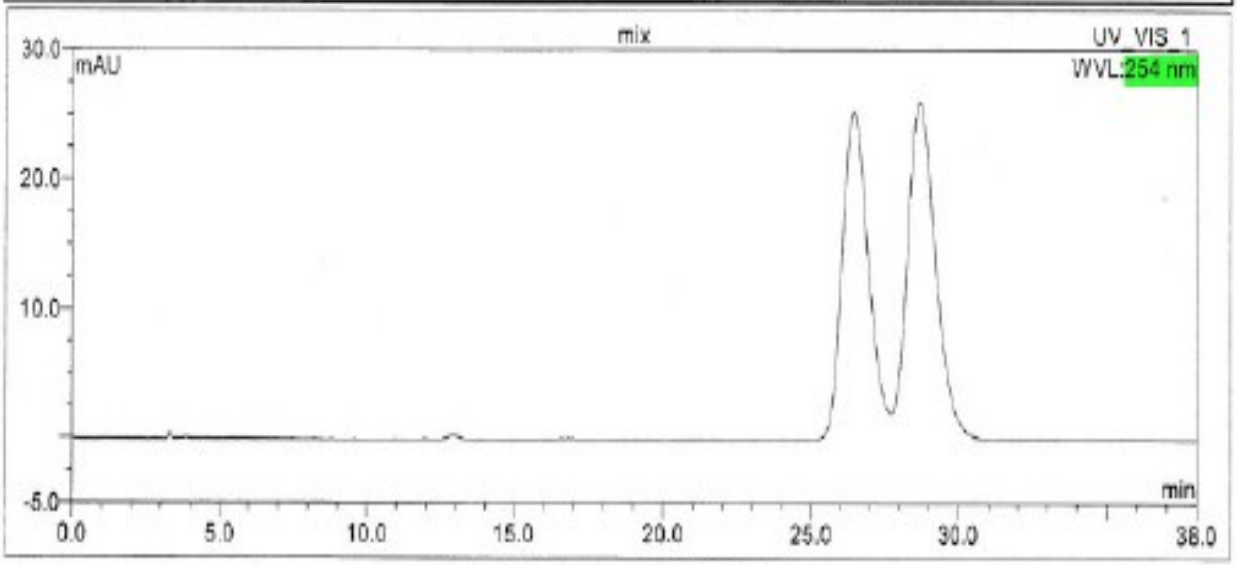


Figure 1: Auto-Scaled Chromatogram

10. DoE Parameter Investigation

Design: 2 Level Factorial including 4 center points

Parameters Varied:	Substrate loading from 150g/L – 250g/L
	NADPH loading from 0.01g/L – 0.1g/L
	Temperature from 30°C – 40°C
	Buffer concentration from 0.04 Mol/L – 0.15 Mol/L
Responses:	Conversion 5.5h
	Conversion 20.5h
	Conversion 28h

3D Surface Plot - Optimum conversion profile at time point 28h: