Electronic Supporting Information

A designer natural deep eutectic solvent to recycle the cofactor in alcohol dehydrogenase-catalysed processes

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I. General methods

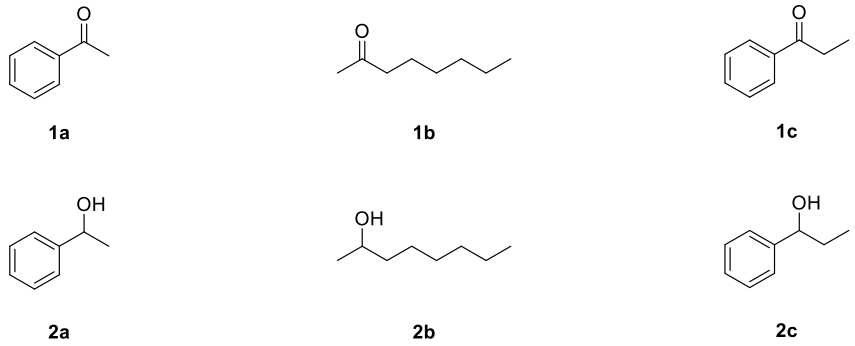
Acetophenone, 1-phenylethanol, propiophenone, 1-phenyl-1-propanol, 2-octanol, 2-octanone, NADPH as enzyme cofactor and all the other chemical reagents were obtained with the highest quality available from Sigma-Aldrich-Fluka (Steinheim, Germany).

Glucose dehydrogenase (GDH-105, 48 U/mg) was obtained from Codexis Inc. (Redwood City, CA, US). Overexpressed ADHs from *Lactobacillus brevis* (LbADH), from *Thermoanaerobacter ethanolicus* (TeSADH), from *Thermoanaerobacter* sp. (ADH-T), from *Sphingobium yanoikuyae* (SyADH) and from *Ralstonia* sp. (RasADH) have been produced following the methodology previously described in the main text.

Gas chromatography (GC) analyses were performed for conversion and enantiomeric excess measurements using:

- an Agilent HP6820 GC chromatograph equipped with a FID detector,
- a CP-Chirasil-Dex-CB column (25 m x 0.25 mm, 0.25 µm as a chiral stationary phase.

II. Analytics



II.1. GC retention times for conversion and enantiomeric excess measurements

GC analyses were carried out for conversion and enantiomeric excess measurements using an Agilent 7890A GC-system and a chiral stationary phase, CP-Chirasil-Dex-CB column (25 m x 0.25 mm, 0.25 µm).

Table S1. GC analyses for conversion measurements of the bioreduction experiments.

Compound	Temperature program[a]	Time (min)
1a	110/0/2.5/120/0/10/200/1	4.8
2a	110/0/2.5/120/0/10/200/1	6.9 (*R*) and 7.1 (*S*)
1b	110/0/2.5/120/0/10/200/1	7.7
2b	110/0/2.5/120/0/10/200/1	7.9
2b[b]	110/0/2.5/120/0/10/200/1	4.4 (*S*) and 4.8 (*R*)
1c	110/0/2.5/120/0/10/200/1	6.2
2c	110/0/2.5/120/0/10/200/1	8.2
2c[b]	110/0/2.5/120/0/10/200/1	6.6 (*S*) and 6.8 (*R*)

[a] Initial temperature (ºC)/time (min)/slope (ºC/min)/temperature (ºC)/time (min)/slope (ºC/min)/final temperature (ºC)/time (min). [b] Alcohol acetylated using DMAP and acetic anhydride.

III. Screening of alcohol dehydrogenases (see Table 1 in the main manuscript)

III.1. General procedure for the bioreduction of acetophenone using LbADH

Lyophilised *E. coli*/LbADH cells (10 mg), 1 mM NADPH (60 µL of a 10 mM stock solution), 1 mM $MgCl_2 \cdot 6H_2O$ (60 µL of a 10 mM stock solution) and glucose dehydrogenase (GDH-105, 3 U) were added into an Eppendorf tube containing acetophenone (**1a**, 25-200 mM) in a mixture of Tris·HCl buffer 50 mM pH 7.5 and NADES ChCl:Glu (1.5:1 mol/mol, 10-50% v/v). The reaction was incubated at 30 °C and 250 rpm for 24 hours. After this time, the mixture was extracted with EtOAc (2 x 500 µL), the organic layers separated by centrifugation (2 min, 13000 rpm), combined and finally dried over Na_2SO_4. Conversion and enantiomeric excess of 1-phenylethanol (**2a**) were determined by GC analysis.

III.2. General procedure for the bioreduction of 2-octanone or propiophenone using TeSADH, ADH-T, SyADH or RasADH

Lyophilised *E. coli*/TeSADH, ADH-T, SyADH or RasADH cells (10 mg), 1 mM NADPH (60 µL of a 10 mM stock solution) and glucose dehydrogenase (GDH-105, 3 U) were added into an Eppendorf tube containing 2-octanone or propiophenone (**1b** or **1c**, 25-200 mM) in a mixture of Tris·HCl buffer 50 mM pH 7.5 and NADES ChCl:Glu (1.5:1 mol/mol, 10-50% v/v). The reaction was incubated at 30 °C and 250 rpm for 24 hours. After this time, the mixture was extracted with ethyl acetate (2 x 500 µL), the organic layers separated by centrifugation (2 min, 13000 rpm), combined and finally dried over Na_2SO_4.

III.3. General procedure for the bioreduction of acetophenone using LbADH and the glucose/GDH system (in the absence of DES)

Lyophilised *E. coli*/LbADH cells (10 mg), 1 mM NADPH (60 µL of a 10 mM stock solution), 1 mM $MgCl_2 \cdot 6H_2O$ (60 µL of a 10 mM stock solution), 240 mM glucose (60 µL of a 2.4 M stock solution) and glucose dehydrogenase (GDH-105, 3 U) were added into an Eppendorf tube containing acetophenone (**1a**, 100 mM) in Tris·HCl buffer 50 mM pH 7.5. The reaction was incubated at 30 °C and 250 rpm for 24 hours. After this time, the mixture was extracted with ethyl acetate (2 x 500 µL), the organic layers separated by centrifugation (2 min, 13000 rpm), combined and finally dried over Na_2SO_4.

III.4. General procedure for the bioreduction of 2-octanone or propiophenone using ADH-T or RasADH and the glucose/GDH system (in the absence of DES)

Lyophilised *E. coli*/ADH-T or RasADH cells (10 mg), 1 mM NADPH (60 µL of a 10 mM stock solution), 240 mM glucose (60 µL of a 2.4 M stock solution) and glucose dehydrogenase (GDH-105, 3 U) were added into an Eppendorf tube containing 2-octanone or propiophenone (**1b** or **1c**, 25-200 mM) in a mixture of Tris·HCl buffer 50 mM pH 7.5. The reaction was incubated at 30 °C and 250 rpm for 24 hours. After this time, the mixture was extracted with ethyl acetate (2 x 500 µL), the organic layers separated by centrifugation (2 min, 13000 rpm), combined and finally dried over Na_2SO_4.

III.5. Extensive optimisation of bioreduction conditions

Table S2. Bioreduction of ketones (control experiments)

Entry	ADH	NADPH	Conversion (%)[a]
1	Lb	1 mM	4
2	Lb	-	2
3	T	1 mM	<1
4	T	-	<1
5	Ras	1 mM	3
6	Ras	-	3

[a] Measured by GC.

Table S3. Bioreduction of ketones using 10-90% v/v ChCl:Glu (see Figure 1 in the main manuscript)

Entry	ADH	NADES (% v/v)	Conversion (%)[a]	*ee* 2a-c (%)[a]
1	Lb	10	>99	>99
2	Lb	20	>99	>99
3	Lb	30	>99	>99
4	Lb	40	>99	>99
5	Lb	50	>99	>99
6	Lb	60	>99	>99
7	Lb	70	92	>99
8	Lb	80	15	>99
9	Lb	90	<1	n.d.
10	T	10	99	>99
11	T	20	99	>99
12	T	30	98	>99
13	T	40	98	>99
14	T	50	97	>99
15	T	60	46	>99
16	T	70	39	>99
17	T	80	5	n.d.
18	T	90	2	n.d.
19	Ras	10	>99	>99
20	Ras	20	>99	>99
21	Ras	30	>99	>99
22	Ras	40	>99	93
23	Ras	50	>99	90
24	Ras	60	>99	90
25	Ras	70	>99	89
26	Ras	80	52	80
27	Ras	90	2	n.d.

[a] Measured by GC.

Table S4. Bioreduction of ketones using different substrate concentrations at 10% v/v ChCl:Glu (see Figure 2A in the main manuscript)

Entry	ADH	[Ketone 1a-c] (mM)	Conversion (%)[a]	*ee* 2a-c (%)[a]
1	Lb	30	>99	>99
2	Lb	40	>99	>99
3	Lb	50	>99	>99
4	Lb	75	>99	>99
5	Lb	100	>99	>99
6	Lb	150	78	>99
7	Lb	200	63	>99
8	T	30	>99	>99
9	T	40	98	>99
10	T	50	98	>99
11	T	75	75	98
12	T	100	58	98
13	T	150	41	97
14	T	200	32	96
15	Ras	30	>99	>99
16	Ras	40	>99	>99
17	Ras	50	>99	>99
18	Ras	75	99	>99
19	Ras	100	83	>99
20	Ras	150	65	>99
21	Ras	200	56	>99

[a] Measured by GC.

Table S5. Bioreduction of ketones using different substrate concentrations at 30% v/v ChCl:Glu (see Figure 2B in the main manuscript)

Entry	ADH	[Ketone 1a-c] (mM)	Conversion (%)[a]	*ee* 2a-c (%)[a]
1	Lb	25	>99	>99
2	Lb	50	>99	>99
3	Lb	100	>99	>99
4	Lb	200	87	>99
5	T	25	>99	>99
6	T	50	81	98
7	T	100	47	97
8	T	200	23	94
9	Ras	25	>99	>99
10	Ras	50	>99	>99
11	Ras	100	95	>99
12	Ras	200	62	>99

[a] Measured by GC.

IV. Semi-preparative bioreductions

IV.1. General procedure for the conversion of acetophenone using LbADH

Acetophenone (100 mg, 100 mM) was added to a mixture of 30% v/v NADES ChCl:Glu (1.5:1 mol/mol, 2.5 mL) and Tris·HCl buffer 50 mM pH 7.5 (5.8 mL). The reaction media was implemented with 1 mM NADPH and 1 mM $MgCl_2 \cdot 6H_2O$. Later on, glucose dehydrogenase (GDH-105, 37 U) and LbADH (150 mg) were added. The reaction was incubated at 30 °C and 250 rpm for 24 hours. After this time, the mixture was extracted with ethyl acetate (2 x 10 mL), the organic layers separated by centrifugation (5 min, 4900 rpm), combined and finally dried over Na_2SO_4. Enantiopure (*R*)-1-phenylethanol (**2a**) was obtained with full conversion and high isolated yield (>99% *ee*, >99% conversion, 78% yield).

IV.2. General procedure for the conversion of 2-octanone using ADH-T

2-Octanone (100 mg, 50 mM) was added to a mixture of 30% v/v NADES ChCl:Glu (1.5:1 mol/mol, 4.68 mL) and Tris·HCl buffer 50 mM pH 7.5 (7.32 mL) implemented with 1 mM NADPH. Afterwards, glucose dehydrogenase (GDH-105, 155 U) and ADH-T (350 mg) were added. The reaction was incubated at 30 °C and 250 rpm for 24 hours. After this time, the mixture was extracted with ethyl acetate (2 x 10 mL), the organic layers separated by centrifugation (5 min, 4900 rpm), combined and finally dried over Na_2SO_4. Enantiopure (*S*)-2-octanol (**2b**) was obtained with full conversion and high isolated yield (>99% *ee*, >99% conversion, 85% yield).

IV.3. General procedure for the conversion of propiophenone using RasADH

Propiophenone (100 mg, 100 mM) was added to a mixture of 30% v/v NADES ChCl:Glu (1.5:1 mol/mol, 2.24 mL) and Tris·HCl buffer 50 mM pH 7.5 (5.2 mL). The reaction media was implemented with 1 mM NADPH and glucose dehydrogenase (GDH-105, 37 U) and RasADH (150 mg) were finally added. The reaction was incubated at 30 °C and 250 rpm for 24 hours. After this time, the mixture was extracted with ethyl acetate (2 x 10 mL), the organic layers separated by centrifugation (5 min, 4900 rpm), combined and finally dried over Na_2SO_4. Enantiopure (*S*)-1-phenyl-1-propanol (**2c**) was obtained with full conversion and high isolated yield (>99% *ee*, >99% conversion, 89% yield).

V. Recycling experiments

Table S6. Bioreduction of ketones in recycling experiments using 25 mM substrate concentrations at 30% v/v ChCl:Glu and extracting with EtOAc (see Figure 3A in the main manuscript)

Entry	ADH	Time	Conversion (%)[a]	*ee* 2a-c (%)[a]
1	Lb	1 h	>99	>99
2	Lb	1 h	20	>99
3	T	1.5 h	>99	>99
4	T	1.5 h	10	94
5	Ras	20 min	>99	>99
6	Ras	20 min	56	>99

[a] Measured by GC.

Table S7. Bioreduction of ketones in recycling experiments using 25 mM substrate concentrations at 30% v/v ChCl:Glu and extracting with pentane using different Tris HCl buffers pH 7.5 (see Figure 3B in the main manuscript)

Entry	Cycle	ADH	Time	Buffer (mM)	Conversion (%)[a]	*ee* 2a-c (%)[a]
1	1	Lb	1 h	50	>99	>99
2	2	Lb	1 h	50	97	>99
3	3	Lb	1 h	50	47	>99
4	4	Lb	1 h	50	30	>99
5	5	Lb	1 h	50	20	>99
6	1	Lb	1 h	200	>99	>99
7	2	Lb	1 h	200	>99	>99
8	3	Lb	1 h	200	82	>99
9	4	Lb	1 h	200	48	>99
10	5	Lb	1 h	200	33	>99
11	1	T	1.5 h	50	>99	>99
12	2	T	1.5 h	50	35	>99
13	3	T	1.5 h	50	17	>99
14	4	T	1.5 h	50	8	>99
16	1	T	1.5 h	200	>99	>99
17	2	T	1.5 h	200	69	>99
18	3	T	1.5 h	200	28	>99
19	4	T	1.5 h	200	18	>99
21	1	Ras	20 min	50	>99	>99
22	2	Ras	20 min	50	99	>99
23	3	Ras	20 min	50	54	>99
24	4	Ras	20 min	50	28	>99
25	5	Ras	20 min	50	17	>99
26	1	Ras	20 min	200	>99	>99
27	2	Ras	20 min	200	>99	>99
28	3	Ras	20 min	200	98	>99
29	4	Ras	20 min	200	77	>99
30	5	Ras	20 min	200	42	>99

[a] Measured by GC.

Table S8. Recycling of ADH-T using Tris·HCl pH 7.5 and 30% v/v NADES.

Entry	Cycle	Conv. (%) buffer 50 mM[a]	Conv. (%) buffer 200 mM[a]	Conv. (%) after ADH filtration[a,b]	Conv. (%) after readjusting pH[a,c]
1	1	>99	>99	>99	>99
2	2	35	69	51	76
3	3	17	28	27	66
4	4	8	18	15	28

[a] In all cases, *ee* values remained excellent (>99% *ee*). [b] Tris·HCl 200 mM was the buffer of choice since it gave better results than the 50 mM buffer. After 1.5 h, the reaction mixture was centrifuged and the aqueous layer and the cells were separated. The aqueous layer was extracted with pentane (2 x 500 µL). After extraction, the aqueous mixture was poured back into the Eppendorf containing the cells and 2-octanone was added. [c] Tris·HCl 200 mM was employed and the same extraction protocol described before was followed. Additionally, the aqueous layer was basified up to pH 7.5 after extracting the product. In all cases conversions were measured by GC.

Table S9. Recycling of LbADH using Tris·HCl pH 7.5 and 30% v/v NADES.

Entry	Cycle	Conv. (%) buffer 50 mM[a]	Conv. (%) buffer 200 mM[a]	Conv. (%) after ADH filtration[a,b]	Conv. (%) after readjusting pH[a,c]
1	1	>99	>99	>99	>99
2	2	97	>99	>99	>99
3	3	47	82	88	>99 (98% *ee*)
4	4	30	48	62	>99 (98% *ee*)
5	5	20	33	45	98 (98% *ee*)

[a] If not specified, *ee* values remained excellent (>99% *ee*). [b] Tris·HCl 200 mM was the buffer of choice since it gave better results than the 50 mM buffer. After 1.5 h, the reaction mixture was centrifuged and the aqueous layer and the cells were separated. The aqueous layer was extracted with pentane (2 x 500 µL). After extraction, the aqueous mixture was poured back into the Eppendorf containing the cells and 2-octanone was added. [c] Tris·HCl 200 mM was employed and the same extraction protocol described before was followed. Additionally, the aqueous layer was basified up to pH 7.5 after extracting the product. In all cases conversions were measured by GC.

Table S10. Recycling of RasADH using Tris·HCl pH 7.5 and 30% v/v NADES.

Entry	Cycle	Conv. (%) buffer 50 mM[a]	Conv. (%) buffer 200 mM[a]	Conv. (%) after ADH filtration[a,b]	Conv. (%) after readjusting pH[a,c]
1	1	>99	>99	>99	>99
2	2	99	>99	99	>99
3	3	54	98	98	>99
4	4	28	77	73	>99
5	5	17	42	42	>99

[a] In all cases, *ee* values remained excellent (>99% *ee*). [b] Tris·HCl 200 mM was the buffer of choice since it gave better results than the 50 mM buffer. After 1.5 h, the reaction mixture was centrifuged and the aqueous layer and the cells were separated. The aqueous layer was extracted with pentane (2 x 500 µL). After extraction, the aqueous mixture was poured back into the Eppendorf containing the cells and 2-octanone was added. [c] Tris·HCl 200 mM was employed and the same extraction protocol described before was followed. Additionally, the aqueous layer was basified up to pH 7.5 after extracting the product. In all cases conversions were measured by GC.

Table S11. Recycling of RasADH using Tris·HCl pH 7.5, 30% v/v NADES and readjusting the pH after extracting the product.

Entry	Cycle	Conv. (%)[a]	*ee* (%)[a]	Yield (%)[b]
1	1	>99	>99	88
2	2	>99	>99	98
3	3	>99	>99	78
4	4	>99	>99	98
5	5	>99	>99	88
6	6	>99	>99	78
7	7	>99	>99	88
8	8	98	>99	n.d.
9	9	92	>99	n.d.
10	10	48	89	n.d.
11	11	26	31	n.d.

[a] Measured by GC. n.d.: not determined. [b] Isolated yield.

VI. EATOS calculations

E-factor calculations (Figures S1 and S2) were performed using the EATOS (v. 1.1) software tool. The work-up protocols have been taking into account for their comparison. Water has not been taken into account for making these calculations.

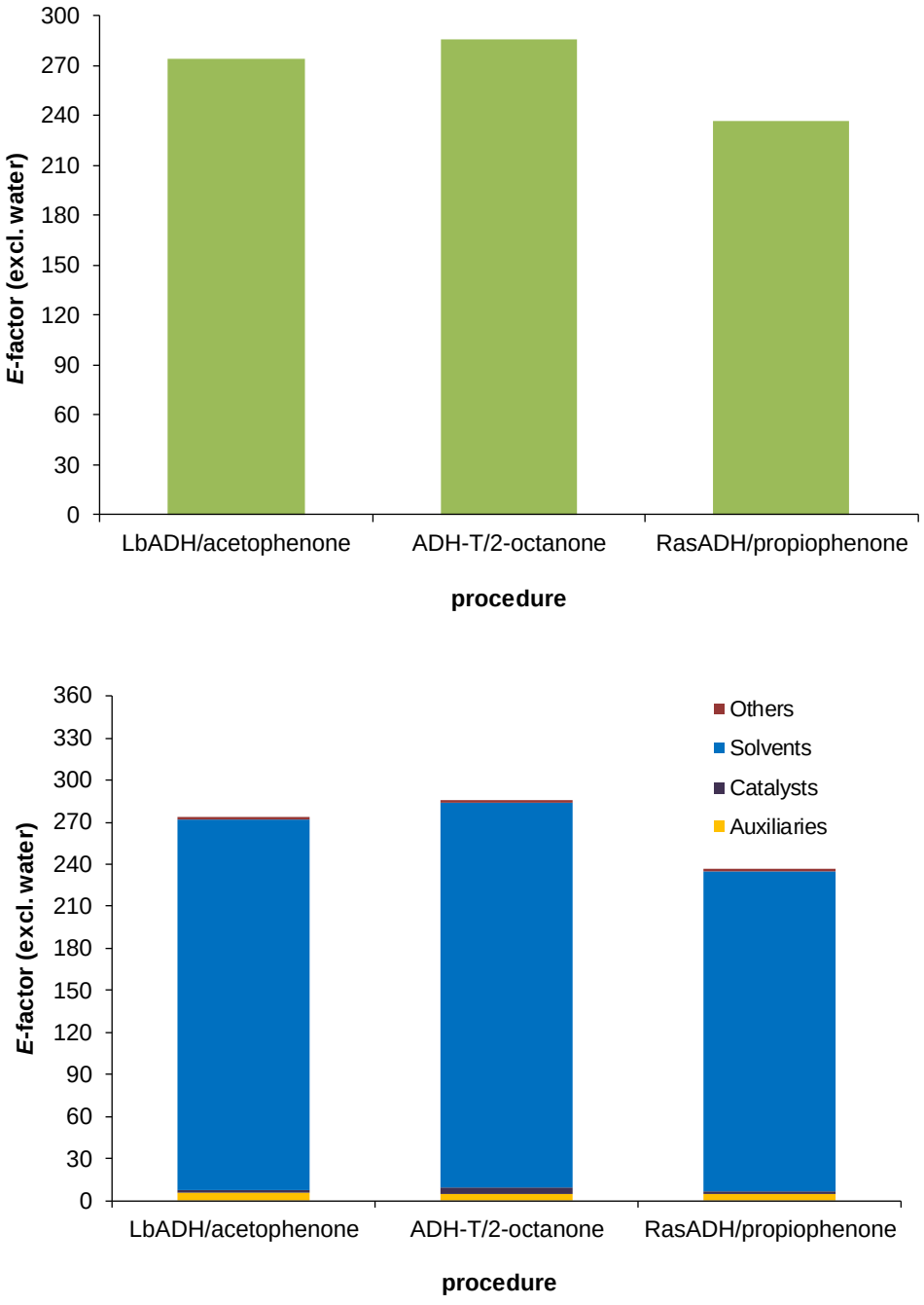
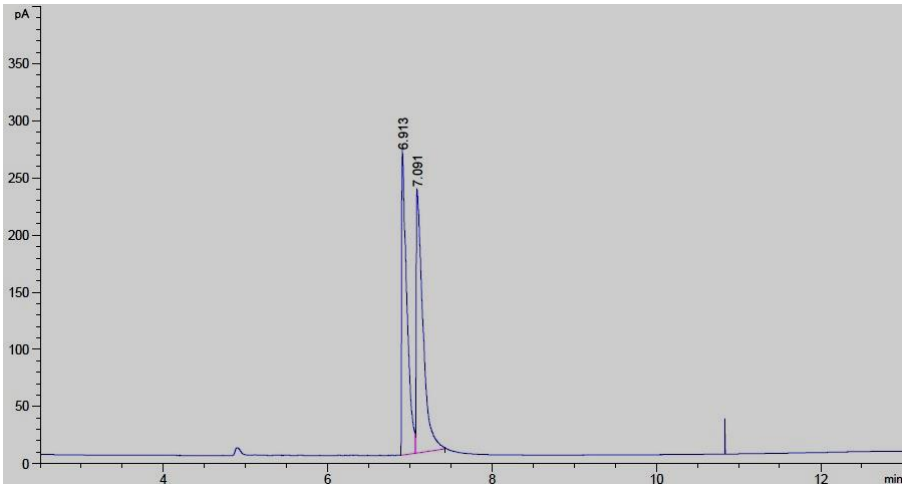


Figure S1. Contribution to *E*-factor (excluding water) for each procedure to reduce acetophenone with LbADH (left), 2-octanone with ADH-T (middle) and propiophenone with RasADH (right).

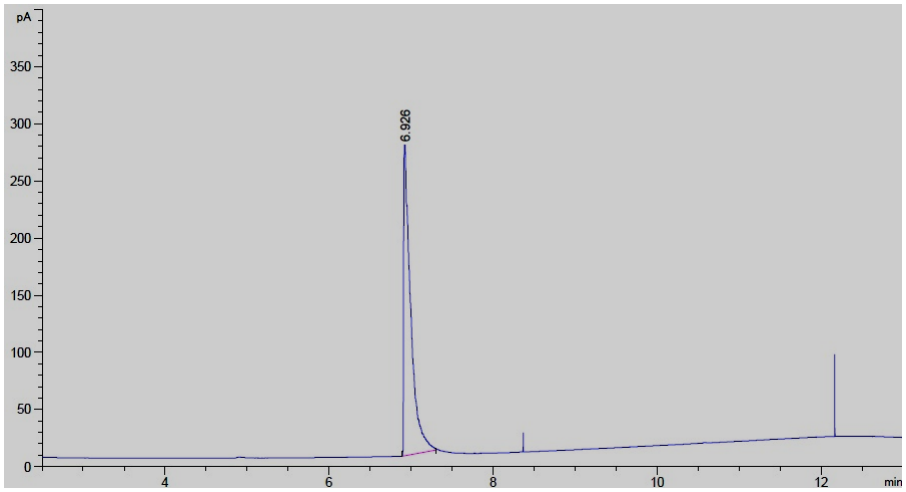
VII. GC chromatograms of racemic and optically active alcohols

Racemic 1-phenylethanol and enantiopure obtained from LbADH-catalysed reactions

GC separation for both enantiomers of 1-phenylethanol (2a)

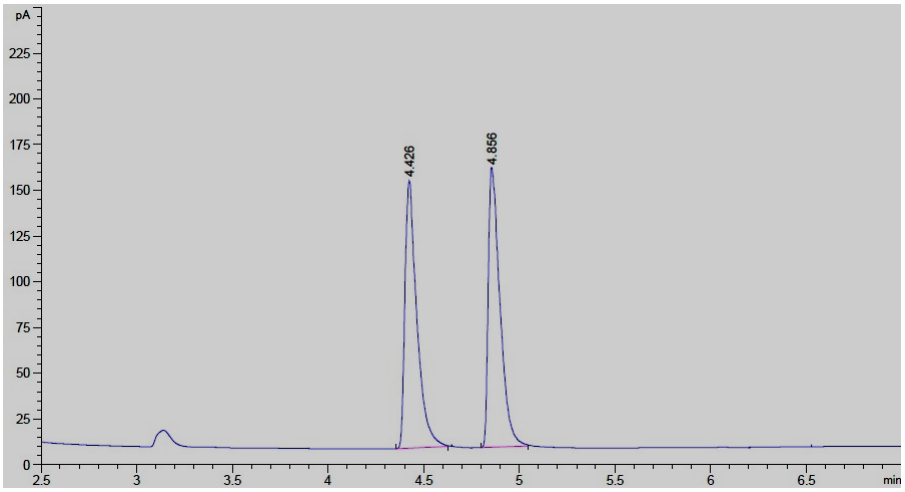


GC chromatogram of alcohol (*R*)-2a in >99% *ee*

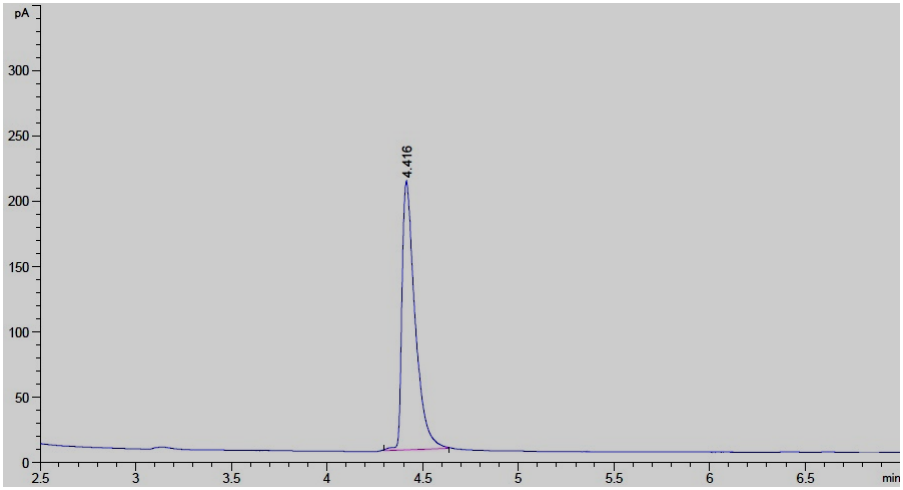


Racemic 2-octanol and enantiopure obtained in ADH-T-catalysed reactions

GC separation for both enantiomers of 2-octanol (2b) derivatised as O-acetylated compound

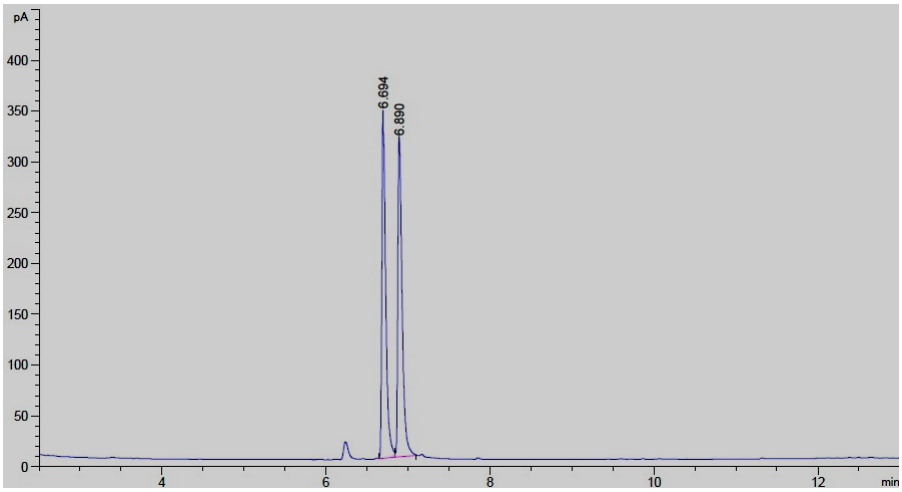


GC chromatogram of alcohol (*S*)-2b in >99% *ee* derivatised as O-acetylated compound

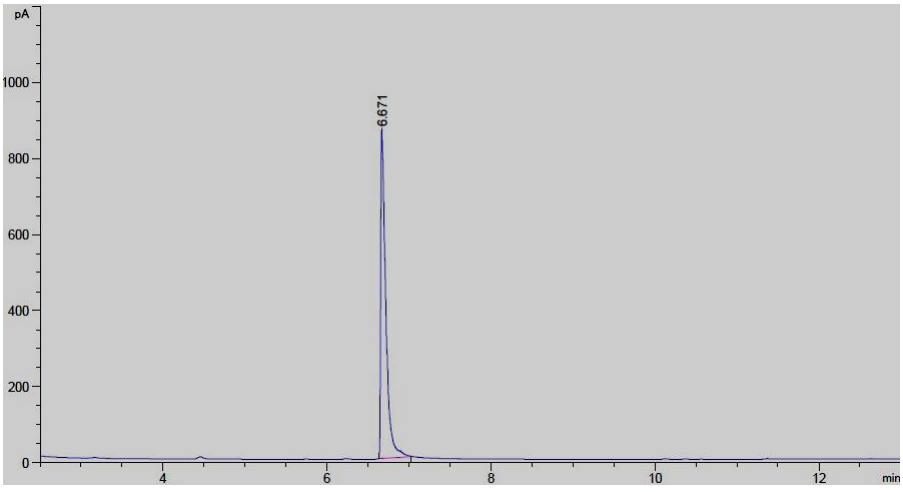


Racemic 1-phenyl-1-propanol and enantiopure obtained in RasADH-catalysed reactions

GC separation for both enantiomers of 1-phenyl-1-propanol (2c) derivatised as O-acetylated compound



GC chromatogram of alcohol (*S*)-2c in >99% *ee* derivatised as O-acetylated compound



VIII. ^{1}H-NMR spectra of ChCl, Glu and mixtures of both components in D_2O

To verify the integrity of the NADES nanostructure a series of ^{1}H-NMR experiments were carried out in D_2O as solvent:

- Glucose and choline chloride as standards
- NADES (ChCl:Glu 1:1.5 mol/mol) 30% and 50% v/v in the deuterated solvent: an important interaction is observed, as many NMR signals exhibit a downfield shift regarding the separate compounds, which implies that at least part of DES is behaving as a pair rather than individual components.
- NADES (ChCl:Glu 1:1.5 mol/mol) 20% and 10% v/v in the deuterated solvent: the components seems to do not interact, so basically ChCl and Glu are in solution and the NADES nanostructure is destroyed. The same was observed when directly mixing ChCl and Glu in the NMR tube (quantities corresponding to 10% v/v).

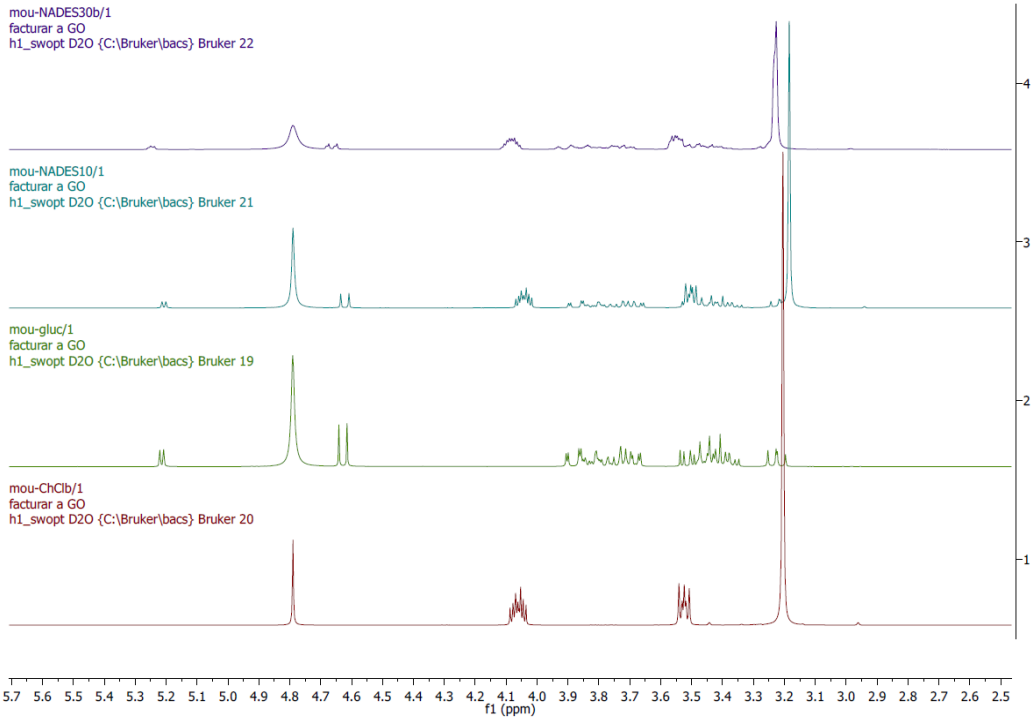


Table S12. ^{1}H-NMR signals of glucose and choline chloride components and different NADES ratios in D_2O solution.

NMR sample[a]	δ_A (ppm)	δ_B (ppm)
Glucose	4.65 and 4.67 (β-D-glucose) 5.25 and 5.26 (α-D-glucose)	
Choline chloride		3.25
Choline chloride and glucose (directly mixed in the NMR tube, 10% v/v)	4.67 and 4.69 (β-D-glucose) 5.26 and 5.27 (α-D-glucose)	3.24
10% v/v NADES	4.67 and 4.69 (β-D-glucose) 5.26 and 5.27 (α-D-glucose)	3.24
20% v/v NADES	4.67 and 4.69 (β-D-glucose) 5.27 and 5.27 (α-D-glucose)	3.25
30% v/v NADES	4.69 and 4.71 (β-D-glucose) 5.28 and 5.29 (α-D-glucose)	3.27
50% v/v NADES	4.73 and 4.75 (β-D-glucose) 5.33 and 5.32 (α-D-glucose)	3.33

[a] Deuterium oxide (D_2O) was employed as solvent in all cases.

- **NMR** (NAV400)

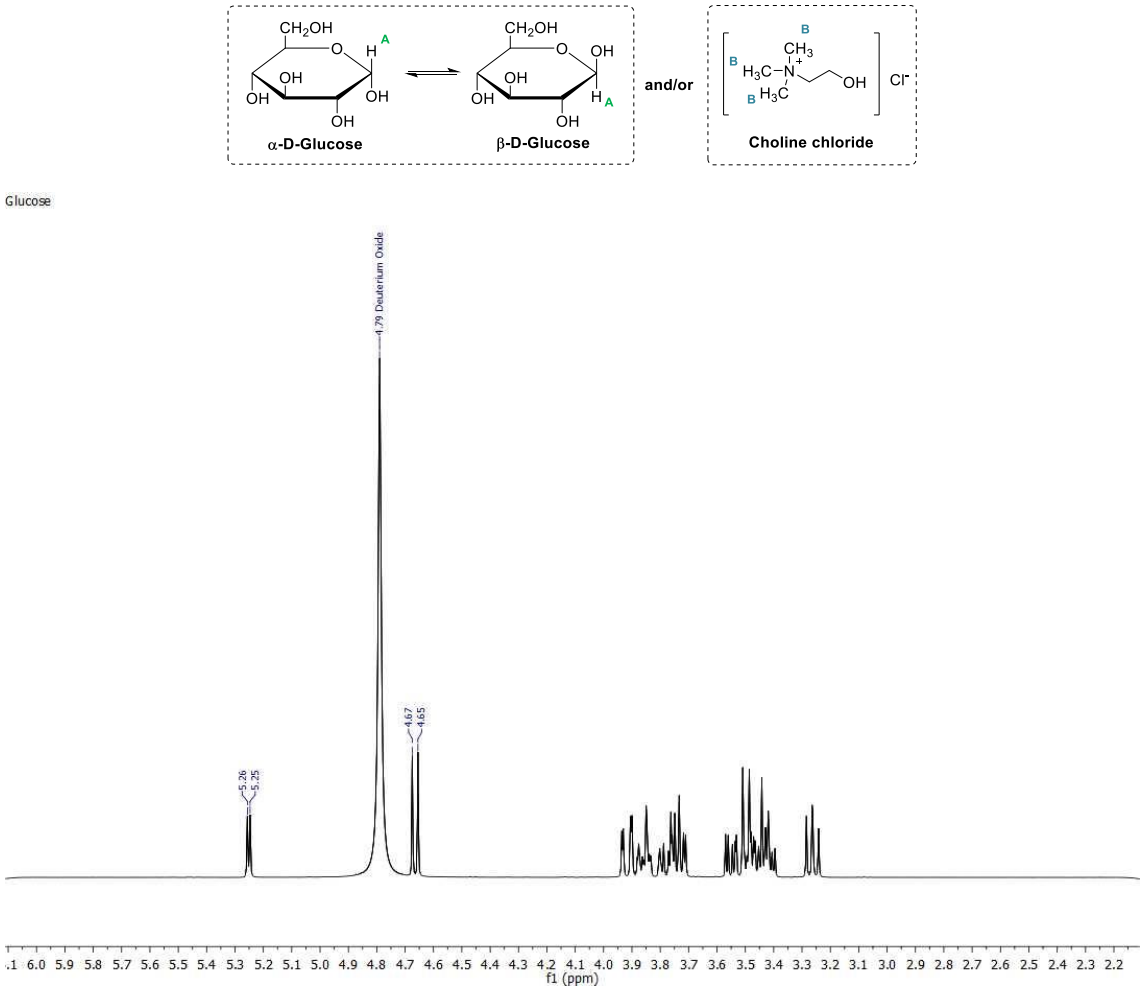


Figure S2. ^{1}H-NMR of glucose in D_2O.

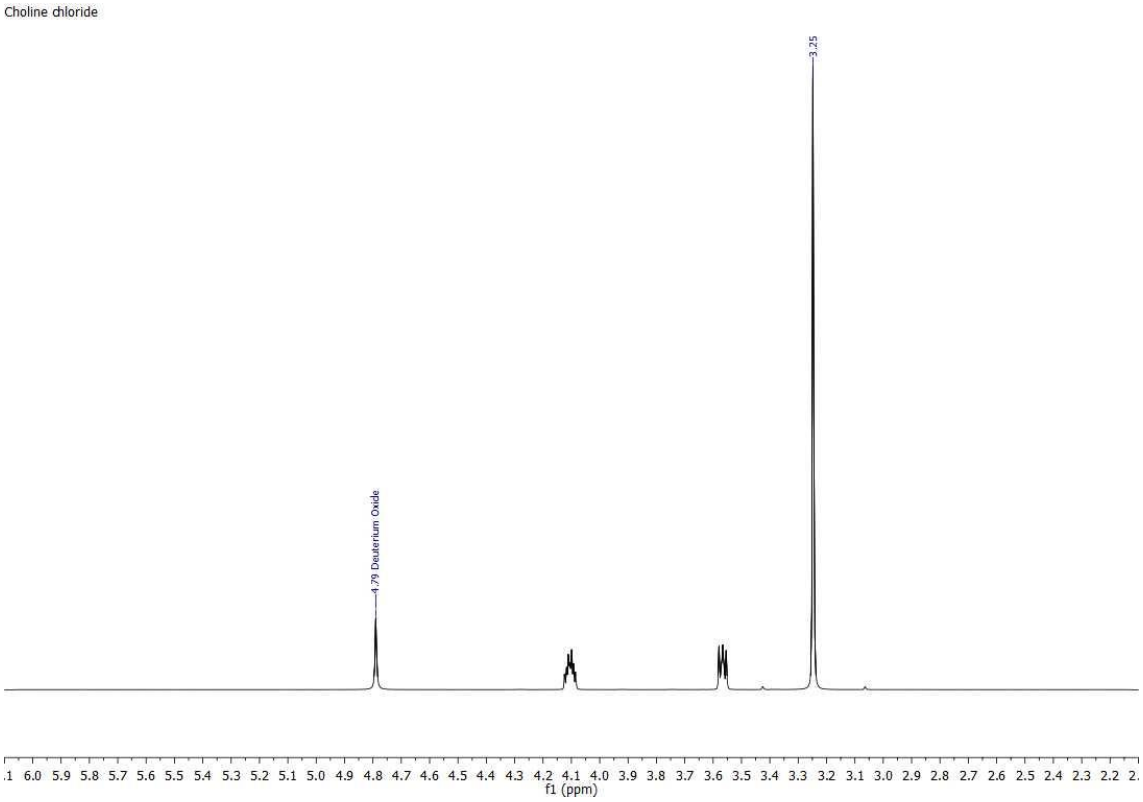


Figure S3. ^{1}H-NMR of choline chloride in D_2O.

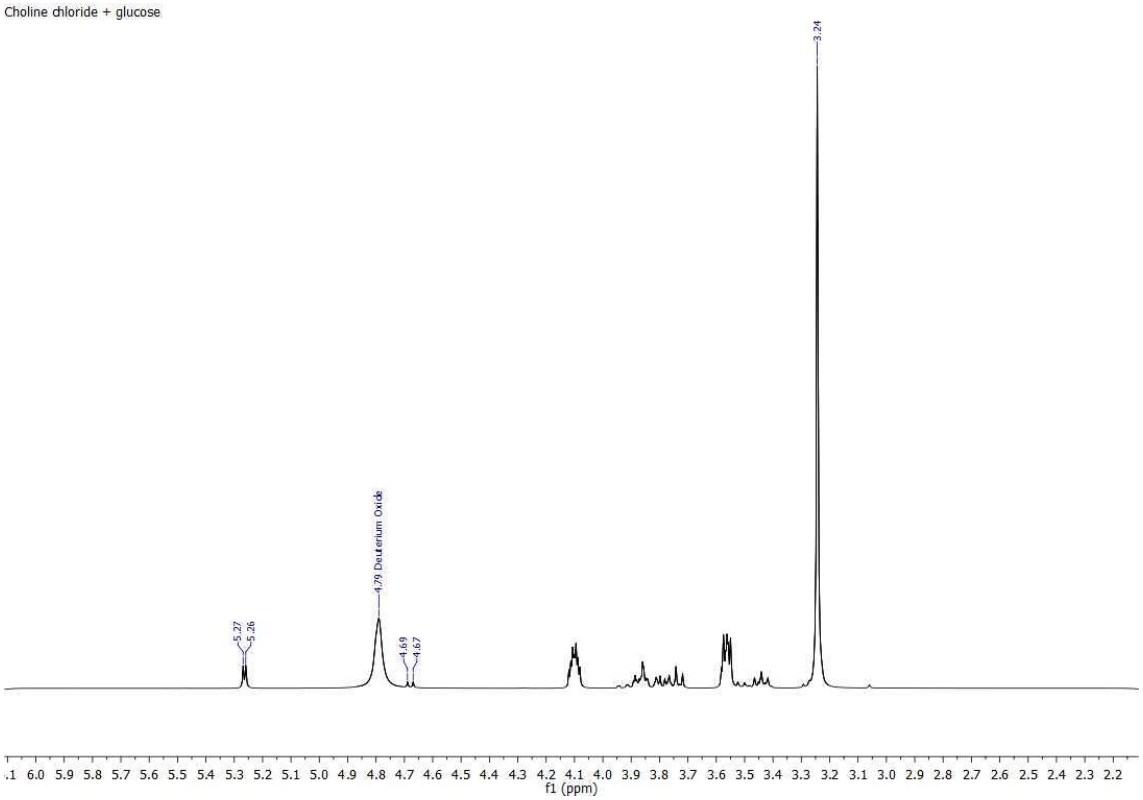


Figure S4. ^{1}H-NMR of glucose and choline chloride (10% v/v) directly mixed in the NMR tube in D_2O.

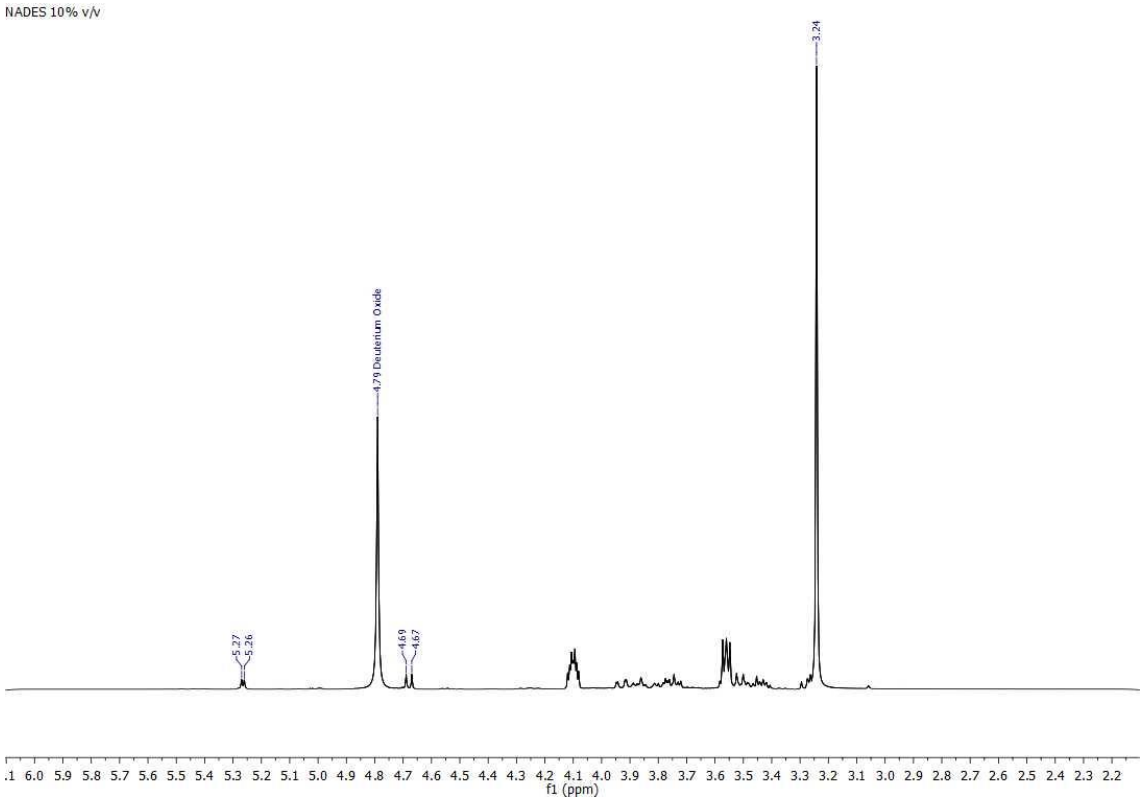


Figure S5. ^{1}H-NMR of NADES (10% v/v) in D_2O.

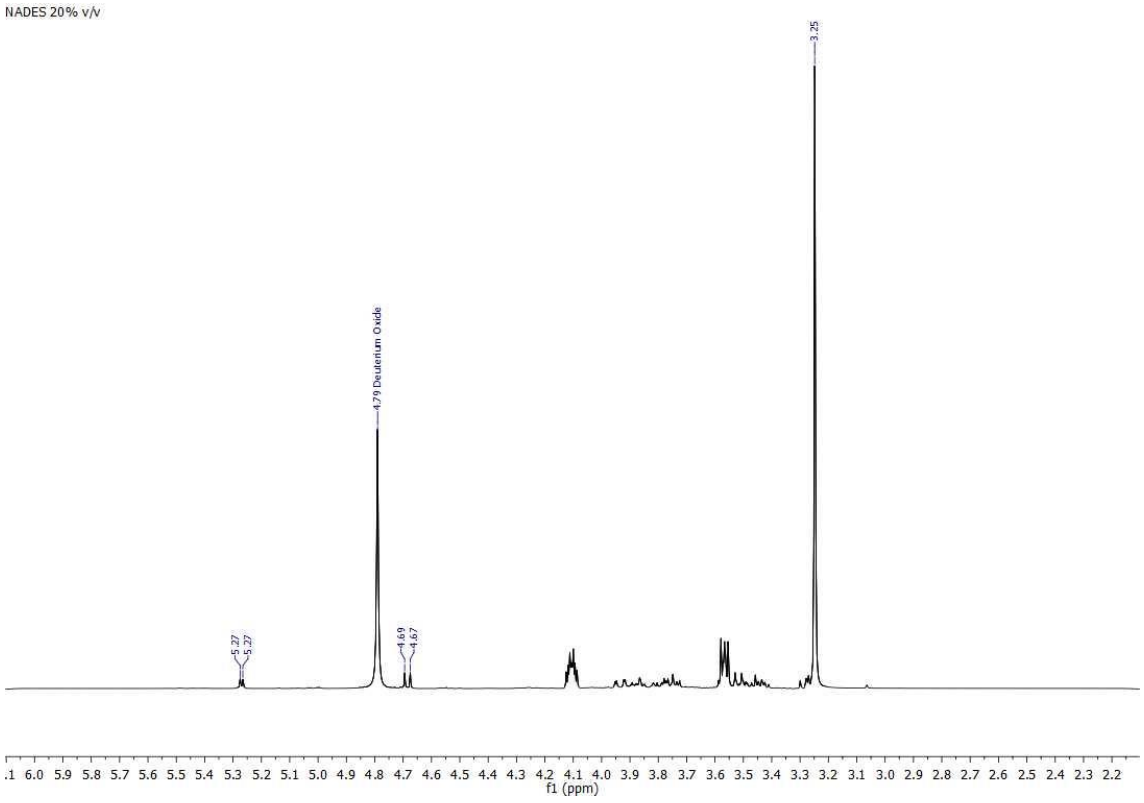


Figure S6. ^{1}H-NMR of NADES (20% v/v) in D_2O.

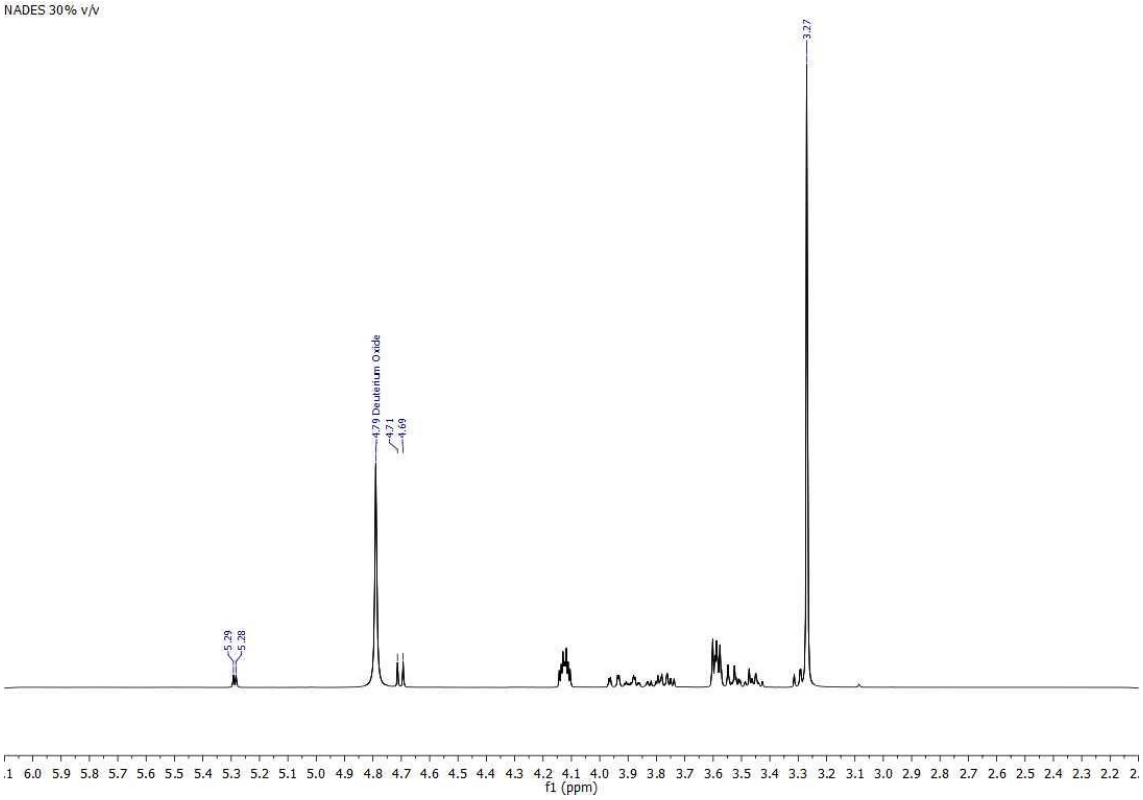


Figure S7. ^{1}H-NMR of NADES (30% v/v) in D_2O.

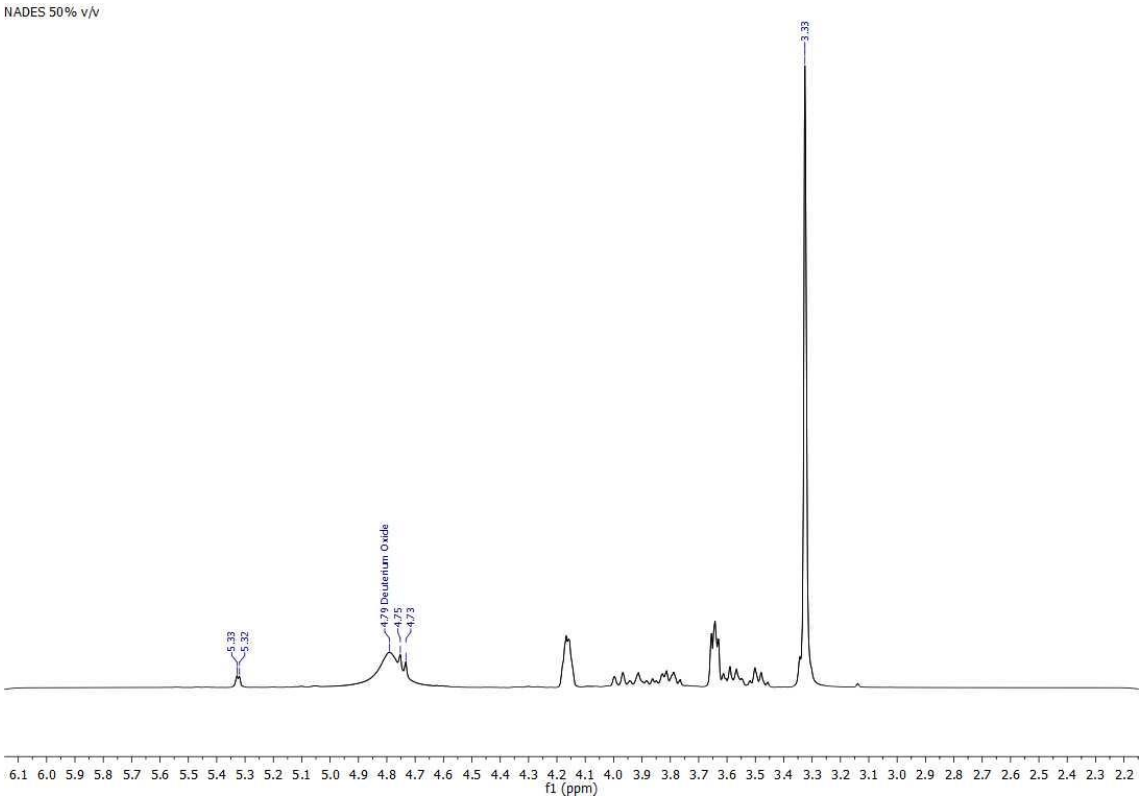


Figure S8. ^{1}H-NMR of NADES (50% v/v) in D_2O.