

Supplementary Materials

Chiral Synthesis of 3-Amino-1-phenylbutane by a Multi- Enzymatic Cascade System

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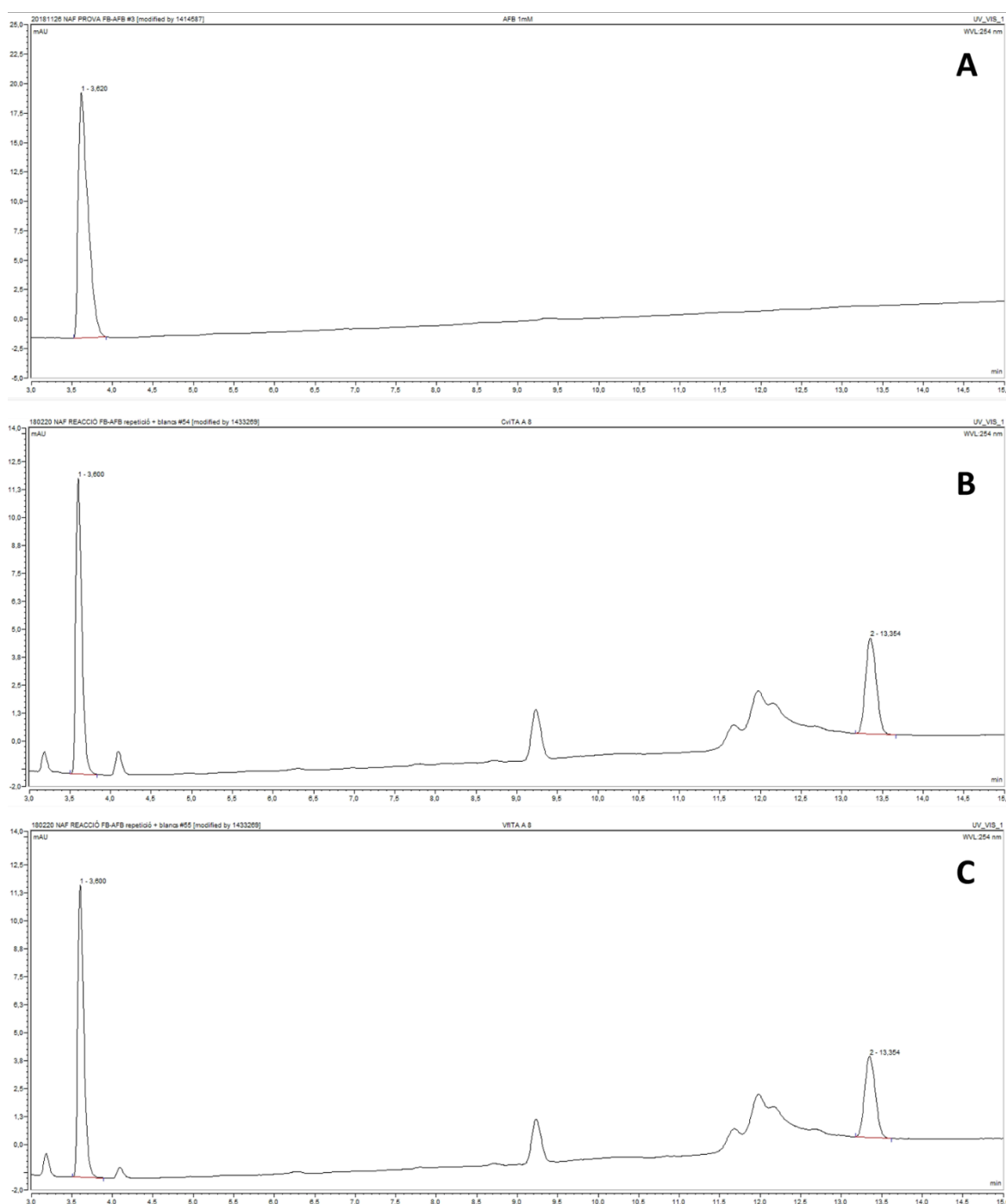


Figure S1. HPLC chromatogram of (A) 1mM commercial 3-APB from Sigma Aldrich (St. Louis, MO, USA), (B) final sample (24 h) of 3-APB synthesis catalyzed by CviTA and PDC and (C) final sample (24h) of 3-APB synthesis catalyzed by CviTA and PDC. Reactions were performed as follows: 5% v v⁻¹ transaminase cell lysate, 25% v v⁻¹ PDC cell lysate, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP), 0.1 mM MgCl₂, 200 mM alanine and 10 mM 4-phenyl-2-butanone (PB) in 150 mM of potassium phosphate buffer, pH 7.5, at 30 °C and 1000 rpm. Analysis were performed in an UltiMate 3000 (Dionex)HPLC equipped with a variable wavelength detector using a CORTECS C18+ 2.7 μ m 4.6 \times 150mm column (Waters Milford, MA, USA). 3-APB retention time: 3.6 min.

Table S1. 4-PB and 3-APB concentrations used for Figure 4A plotting. Reaction conditions: 30°C; pH 7.5; 4-PB 10 mM; L-Alanine 200 mM; CviTA 5% v v⁻¹; PDC 25% v v⁻¹; 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	4-PB	4-PB	4-PB	4-PB	3-APB	3-APB	3-APB	3-APB
	Sample 1 (mM)	Sample 2 (mM)	Average (mM)	Error (mM)	Sample 1 (mM)	Sample 2 (mM)	Average (mM)	Error (mM)
0	9.65	9.65	9.65	0.00	0.00	0	0	0.0
0.5	6.34	6.19	6.27	0.07	3.92	2.85	3.38	0.53
1	5.01	4.60	4.81	0.20	5.22	3.78	4.50	0.72
2	3.69	4.34	4.02	0.33	6.25	4.83	5.54	0.71
4	2.79	3.56	3.18	0.39	6.97	5.20	6.08	0.89
8	2.40	2.99	2.70	0.30	7.18	5.22	6.20	0.98

Table S2. 4-PB and 3-APB concentrations used for Figure 4A plotting. Reaction conditions: 30°C; pH 7.5; 4-PB 10 mM; L-Alanine 200 mM; CviTA 5% v v⁻¹; 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	4-PB	4-PB	4-PB	4-PB	3-APB	3-APB	3-APB	3-APB
	Sample 1 (mM)	Sample 2 (mM)	Average (mM)	Error (mM)	Sample 1 (mM)	Sample 2 (mM)	Average (mM)	Error (mM)
0	11.23	8.17	9.70	1.53	0.00	0.00	0.00	0
0.5	10.02	9.81	9.92	0.10	0.66	0.70	0.68	0.02
1	9.44	9.58	9.51	0.07	0.62	0.71	0.66	0.04
2	9.12	8.83	8.97	0.14	0.59	0.75	0.67	0.08
4	8.33	8.02	8.18	0.15	0.51	0.66	0.59	0.08
8	7.59	7.06	7.33	0.26	0.58	0.58	0.58	0.00

Table S3. 4-PB and 3-APB concentrations used for Figure 4C plotting. Reaction conditions: 30°C; pH 7.5; 4-PB 10 mM; L-Alanine 200 mM; VfiTA 5% v v⁻¹; PDC 25% v v⁻¹; 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	4-PB	4-PB	4-PB	4-PB	3-APB	3-APB	3-APB	3-APB
	Sample 1 (mM)	Sample 2 (mM)	Average (mM)	Error (mM)	Sample1 (mM)	Sample 2 (mM)	Average (mM)	Error (mM)
0	9.73	9.75	9.74	0.01	0.00	0.00	0.00	0.0
0.5	9.10	7.03	8.06	1.03	1.55	1.17	1.36	0.19
1	7.63	5.66	6.65	0.98	2.91	2.80	2.85	0.06
2	5.48	4.42	4.95	0.53	4.81	4.19	4.50	0.31
4	3.94	3.34	3.64	0.30	6.10	5.07	5.58	0.51
8	3.11	2.53	2.82	0.29	6.56	5.29	5.92	0.64

Table S4. 4-PB and 3-APB concentrations used for Figure 4C plotting. Reaction conditions: 30°C; pH 7.5; 4-PB 10 mM; L-Alanine 200 mM; VfiTA 5% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	4-PB Sample 1 (mM)	4-PB Sample 2 (mM)	4-PB Average (mM)	4-PB Error (mM)	3-APB Sample 1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	11.42	8.49	9.96	1.47	0.00	0.00	0.00	0.0
0.5	10.48	10.03	10.26	0.22	0.55	0.70	0.63	0.07
1	9.98	9.76	9.87	0.11	0.65	0.81	0.73	0.08
2	9.33	9.29	9.31	0.02	0.77	0.87	0.82	0.05
4	8.92	8.21	8.56	0.35	0.89	0.92	0.90	0.01
8	7.79	7.29	7.54	0.25	0.96	1.06	1.01	0.05

Table S5. 3-APB concentration used for Figure 5A plotting. Reaction conditions: 15°C, pH 7.5; 4-PB 10 mM; L-Alanine 200 mM; CviTA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal 5' phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	3-APB Sample 1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	0.00	0.00	0.00	0.0
1	2.58	2.54	2.56	0.02
2	4.14	4.16	4.15	0.01
4	5.79	5.83	5.81	0.02
8	6.93	6.87	6.90	0.03
24	7.63	7.73	7.68	0.05

Table S6. 3-APB concentration used for Figure 5A plotting. Reaction conditions: 30°C, pH 7.5; 4-PB 10 mM; L-Alanine 200 mM; CviTA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	3-APB Sample 1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	0.00	0.00	0.00	0.0
0.5	3.92	2.85	3.38	0.53
1	5.22	3.78	4.50	0.72
2	6.25	4.83	5.54	0.71
4	6.97	5.20	6.08	0.89
8	7.18	5.22	6.20	0.98

Table S7. 3-APB concentration used for Figure 5A plotting. Reaction conditions: 40°C, pH 7.5; 4-PB 10 mM; L-Alanine 200 mM; CviTA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	3-APB Sample 1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	0	0	0	0
0.5	3.53	3.67	3.60	0.07
1	4.08	4.26	4.17	0.09
2	4.29	4.80	4.55	0.26
4	3.81	4.49	4.15	0.34
8	2.54	3.62	3.08	0.54

Table S8. 3-APB concentration used for Figure 5C plotting. Reaction conditions: 15°C, pH 7.5; 4-PB 10 mM; L-Alanine 200 mM; VfiTA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	3-APB Sample 1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	0	0	0	0
1	0.16	0.18	0.17	0.01
2	0.37	0.43	0.40	0.03
4	0.98	1.08	1.03	0.05
8	2.03	2.35	2.19	0.16
24	4.63	5.32	4.98	0.34
30	5.00	5.92	5.46	0.46
48	5.42	6.42	5.92	0.50

Table S9. 3-APB concentration used for Figure 5C plotting. Reaction conditions: 30°C, pH 7.5; 4-PB 10 mM; L-Alanine 200 mM; VfiTA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	3-APB Sample 1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	0	0	0	0
0.5	1.55	1.17	1.36	0.19
1	2.91	2.79	2.85	0.06
2	4.81	4.19	4.50	0.31
4	6.10	5.07	5.58	0.51
8	6.56	5.28	5.92	0.64

Table S10. 3-APB concentration used for Figure 5C plotting. Reaction conditions: 40°C, pH 7.5; 4-PB 10 mM; L-Alanine 200 mM; VfiTA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	3-APB Sample 1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	0	0	0	0
0.5	2.83	2.99	2.91	0.08
1	3.89	3.77	3.83	0.06
2	4.46	4.14	4.30	0.16
4	4.30	3.49	3.90	0.40
8	2.75	1.85	2.30	0.45

Table S11. 3-APB concentration used for Figure 6A plotting. Reaction conditions: L-Alanine 200 mM, 30°C; pH 7.5; 4-PB 10 mM; CviTA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	3-APB Sample1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	0	0	0	0
0.5	3.92	2.85	3.38	0.53
1	5.22	3.78	4.50	0.72
2	6.25	4.83	5.54	0.71
4	6.97	5.20	6.08	0.89
8	7.18	5.22	6.20	0.98

Table S12. 3-APB concentration used for Figure 6A plotting. Reaction conditions: L-Alanine 400 mM, 30°C; pH 7.5; 4-PB 10 mM; CviTA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	3-APB Sample 1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	0	0	0	0
0.5	3.49	4.10	3.79	0.30
1	4.54	5.44	4.99	0.45
2	5.68	6.12	5.90	0.22
4	6.29	6.28	6.29	0.01
8	5.87	6.57	6.22	0.35

Table 13. 3-APB concentration used for Figure 6A plotting. Reaction conditions: L-Alanine 600 mM, 30°C; pH 7.5; 4-PB 10 mM; CviTA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	3-APB Sample 1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	0.22	0.12	0.17	0.05
0.5	4.17	4.72	4.44	0.27
1	5.41	6.01	5.71	0.30
2	6.55	6.63	6.60	0.04
4	6.68	6.61	6.65	0.03
8	6.93	6.82	6.88	0.06

Table S14. 3-APB concentration used for Figure 6C plotting. Reaction conditions: L-Alanine 200 mM, 30°C; pH 7.5; 4-PB 10 mM; VfiTA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	3-APB Sample 1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	0	0	0	0
0.5	1.55	1.17	1.36	0.19
1	2.91	2.79	2.85	0.06
2	4.81	4.19	4.50	0.31
4	6.10	5.07	5.58	0.51
8	6.56	5.28	5.92	0.64

Table S15. 3-APB concentration used for Figure 6C plotting. Reaction conditions: L-Alanine 400 mM, 30°C; pH 7.5; 4-PB 10 mM; VfiTA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	3-APB Sample 1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	0	0	0	0
0.5	1.23	1.58	1.41	0.17
1	2.50	3.31	2.90	0.40
2	4.60	5.63	5.11	0.52
4	6.03	6.47	6.25	0.22
8	6.35	6.85	6.60	0.25

Table S16. 3-APB concentration used for Figure 6C plotting. Reaction conditions: L-Alanine 600 mM, 30°C; pH 7.5; 4-PB 10 mM; VflTA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂.

Time (h)	3-APB Sample 1 (mM)	3-APB Sample 2 (mM)	3-APB Average (mM)	3-APB Error (mM)
0	0	0	0	0
0.5	1.17	1.87	1.52	0.35
1	2.79	3.86	3.32	0.53
2	5.25	5.65	5.45	0.20
4	6.48	6.82	6.65	0.17
8	6.91	7.26	7.08	0.18

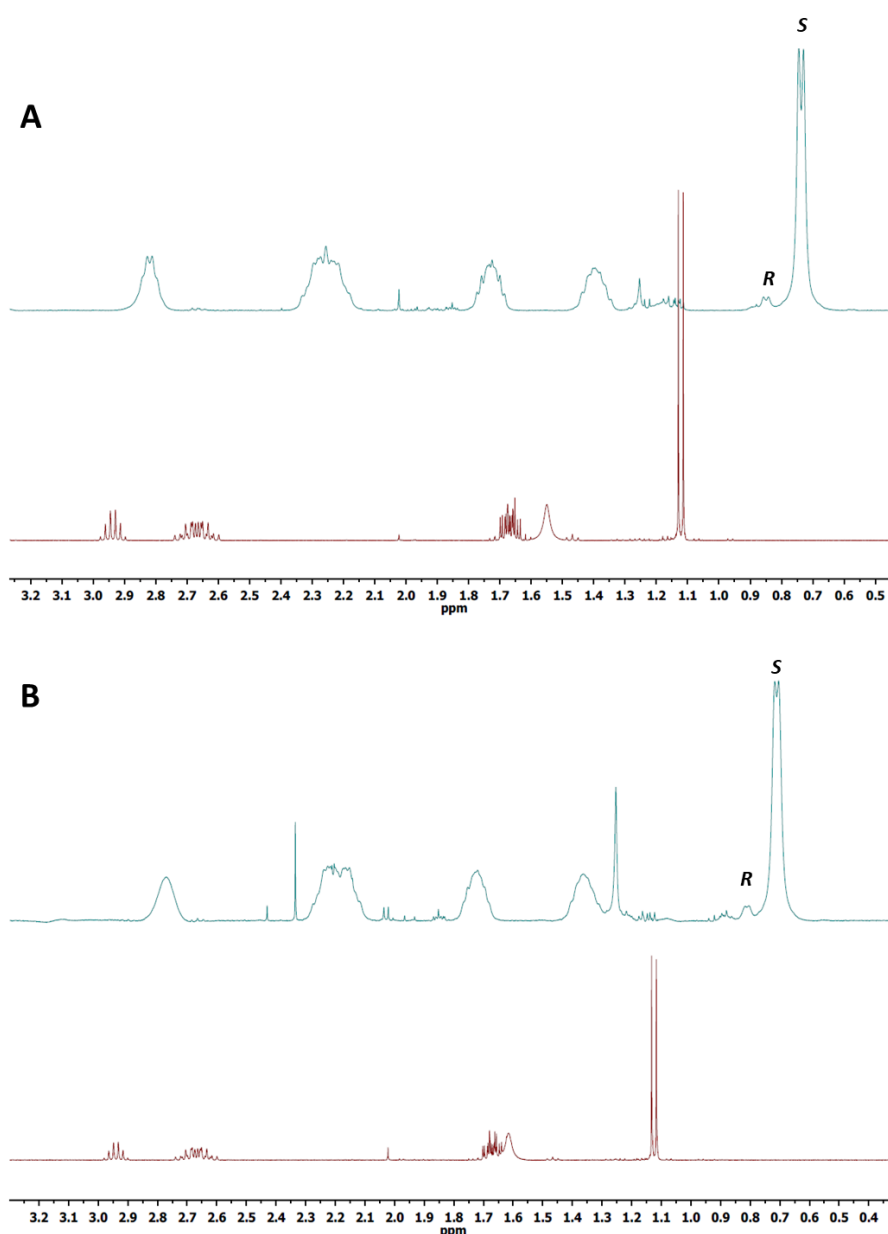


Figure S2. Results on nuclear magnetic resonance (NMR) for 3-APB obtained from reactions catalyzed by A) CviTA-PDC and B) VfiTA-PDC before adding chiral solvating agent (red line) and after adding chiral solvating agent (green line). Reaction conditions: 30°C; pH 7.5; 4-PB 10 mM; L-Alanine 200 mM; TA 5% v v⁻¹; PDC 25% v v⁻¹, 1 mM pyridoxal-5'-phosphate (PLP), 0.1 mM thiamine pyrophosphate (TPP) and 0.1 mM MgCl₂. NMR was performed using (R)-(-)-1,1'-binaphthyl-2,2'-hydrogenphosphate (BHP) as, chiral solvating agent (CSA). The ¹H-NMR spectra were acquired with a Bruker AVANCE-III 600 MHz NMR Spectrometer (Bruker Biospin, Rheinstetten, Germany) in CDCl₃ at 298 K.