Supporting Information

for

Chemoenzymatic Deracemization of Lisofylline Catalyzed by (Laccase/TEMPO)-Alcohol Dehydrogenase System

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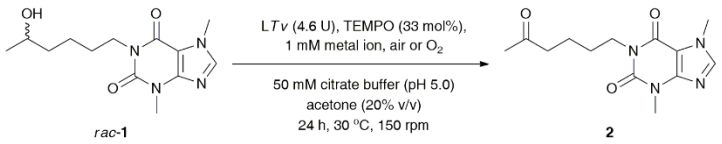
Table S1. Effect of metal ions on laccase/TEMPO-catalyzed oxidation of lisofylline (*rac*-**1**).



Entry	Metal ion[a]	Inorganic salt	Conv.[b] (%)
1	-	-	17
2	-[c]	-	16
3	Mg^{2+}	$MgSO_4$	17
4	Fe^{2+}	$FeCl_2\times4H_2O$	17
5	Fe^{2+}	$FeSO_4\times7H_2O$	15
6	Co^{2+}	$CoCl_2\times6H_2O$	13
7	Zn^{2+}	$ZnSO_4\times7H_2O$	15
8	Cu^{2+}	$CuSO_4\times5H_2O$	17

[a] Reaction conditions: *rac*-**1** (23 mg, 0.08 mmol, 50 mM final conc.), *T. versicolor* laccase (L*Tv*, 7 mg, 4.6 U), TEMPO (4.1 mg, 33% mol), inorganic salt (1 mM final conc.), oxygenated citrate buffer (50 mM, pH 5.0), acetone (20% v/v), 24 h, 30 °C, stirring in an open-to-air test vial (150 rpm, magnetic stirrer). [b] Conversion values (%) (i.e., consumption of substrate *rac*-**1**) were determined by GC analyses after derivatization of crude mixture with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) as a silylating reagent. [c] With additional O_2 bubbling.

Description:

The reaction mixtures performed in citrate buffer (50 mM, pH 5.0), acetone (20% v/v) for 24 h at 30 °C were supplemented with 1 mM final conc. of $MgSO_4$, $FeCl_2\times4H_2O$, $FeSO_4\times7H_2O$, $CoCl_2\times6H_2O$, $ZnSO_4\times7H_2O$, and $CuSO_4\times5H_2O$ as a source of each of the listed metal ions. The control reactions were assayed without added metal ions and in two variants with aerial oxygen as well as under O_2-atmosphere incorporated through an O_2-filled balloon (**Table S1**, entries 1 and 2). The results indicated that there was no positive influence of metal ions on the activity of L*Tv*/TEMPO system in tested reactions.

The addition of Mg^{2+}, Fe^{2+}, Fe^{2+}, Zn^{2+}, and Cu^{2+} was found to enhance the laccase-mediated oxidation almost at the same level (15–17% conv.) in the majority of cases. On the contrary, a negligible decrease in the conversion of *rac*-**1** (13% conv.) was observed for the reactions incubated with $CoCl_2\times6H_2O$. This deterioration of activity is in line with other reports that have demonstrated that Co^{2+} ions slightly reduce the laccase activity due to the unfolding of the enzyme. [please see: (a) S. Afreen, T. N. Shamsi, M. A. Baig, N. Ahmad, S. Fatima, M. I. Qureshi, M. I. Hassan, T. Fatma, *PLoS One*, 2017, **12**, e0175144; (b) M. M. Atalla, H. K. Zeinab, R. H. Eman, A. Y. Amani, A. A. Abeer, *Saudi J. Biol. Sci.*, 2013, **20**, 373–381.]

Table S2. Analytical separation conditions of compounds by GC column.

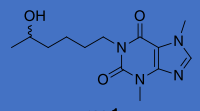
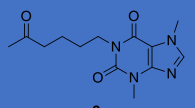
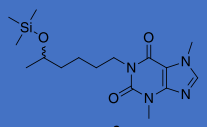
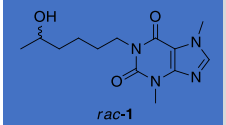
Compound	Temperature program [°C]	Retention time [min]
 *rac*-1	260 (isothermal)	9.47
 2		9.75
 *rac*-3		7.28

Table S3. HPLC analytical separation conditions of racemic lisofylline (*rac*-1) by Chiralpak AD-H (Daicel®) column.

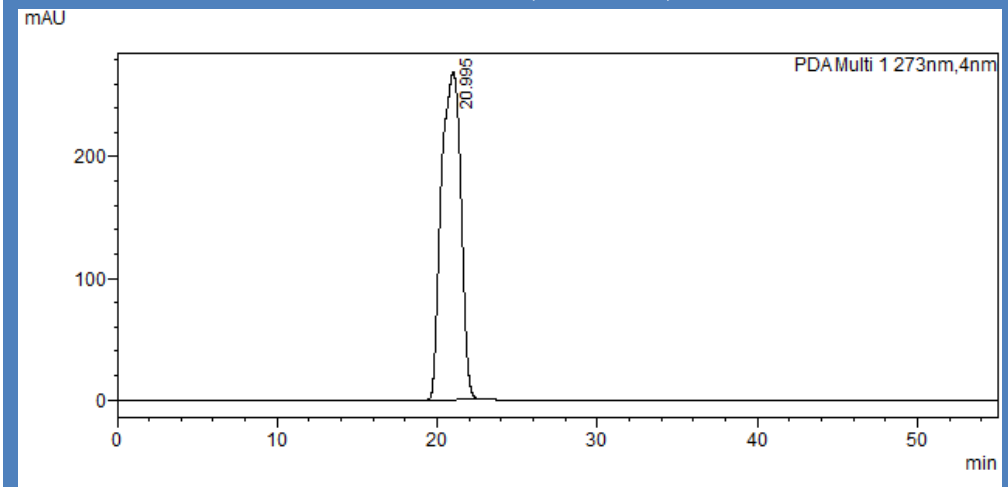
Compound	HPLC Column	Mobile Phase *n*-Hexane/IPA/DEA [v/v/v]	Flow Rate [mL/min]	Detection [nm] (Temperature [°C])	Retention Time [min]
 *rac*-1	Chiralpak AD-H	78:22[a]	1.0	273 (25)	30.763 (*R*) and 33.806 (*S*)
		78:22:0.1[a]	1.0	273 (25)	29.874 (*R*) and 32.738 (*S*)
		78:22[b]	1.0	273 (25)	26.056 (*R*) and 28.875 (*S*)

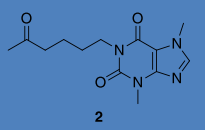
[a] Performed on a Shimadzu Nexera-*i* (LC-2040C 3D) equipped with a photodiode array detector (PAD).

[b] Performed on a Shimadzu CTO-10ASV chromatograph equipped with STD-20A UV detector.

HPLC of 2 on Chiralpak AD-H at 25 °C

HPLC conditions: *n*-hexane-2-PrOH (78:22, v/v); f=1.0 mL/min; λ=273 nm; *p*=5.7 MPa





Peak Table

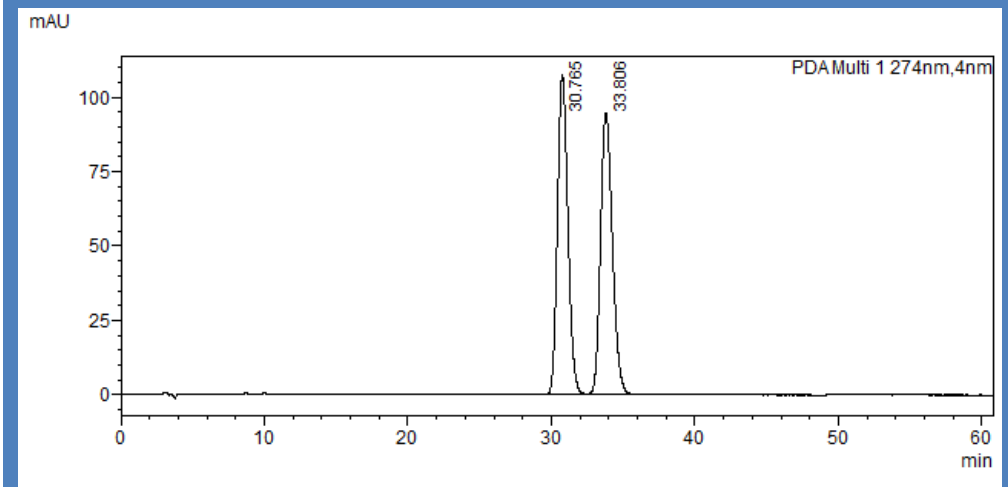
PDA Ch1 273nm

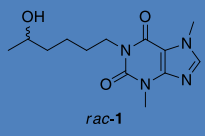
Peak#	Ret. Time	Area	Height	Height%	Area%
1	20.995	22973318	269160	100.000	100.000
Total		22973318	269160	100.000	100.000

HPLC analytical separation for both enantiomers of *rac*-1 on Chiralpak AD-H at 25 °C

[Performed on a Shimadzu Nexera-*i* (LC-2040C 3D) equipped with a photodiode array detector (PAD)]

HPLC conditions: *n*-hexane-2-PrOH (78:22, v/v); f=1.0 mL/min; λ=273 nm; *p*=5.7 MPa





Peak Table

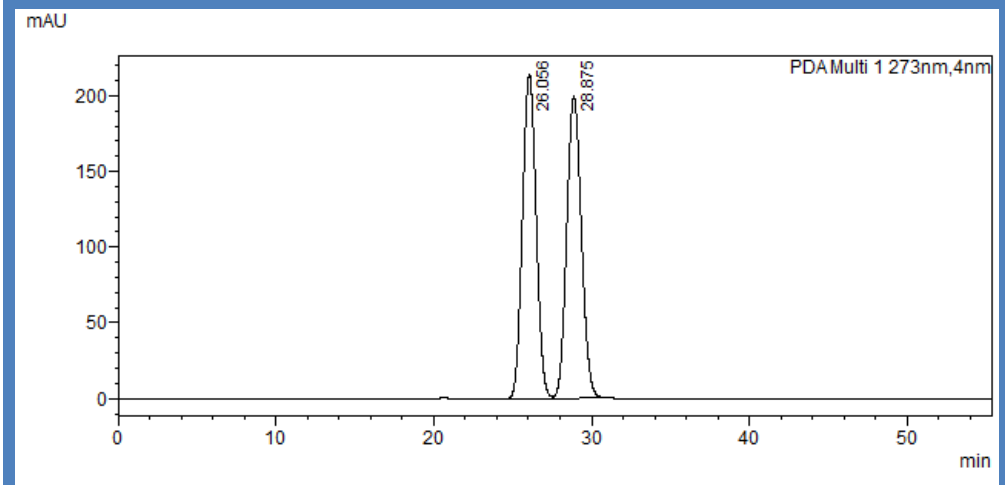
PDA Ch1 274nm

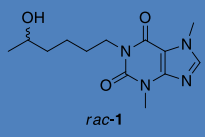
Peak#	Ret. Time	Area	Height	Height%	Area%
1	30.765	5421808	107287	53.164	50.074
2	33.806	5405725	94517	46.836	49.926
Total		10827532	201805	100.000	100.000

HPLC analytical separation for both enantiomers of *rac*-1 on Chiralpak AD-H at 25 °C

[Performed on a Shimadzu CTO-10ASV chromatograph equipped with STD-20A UV detector]

HPLC conditions: *n*-hexane-2-PrOH (78:22, v/v); f=1.0 mL/min; λ=273 nm; *p*=5.5 MPa





Peak Table

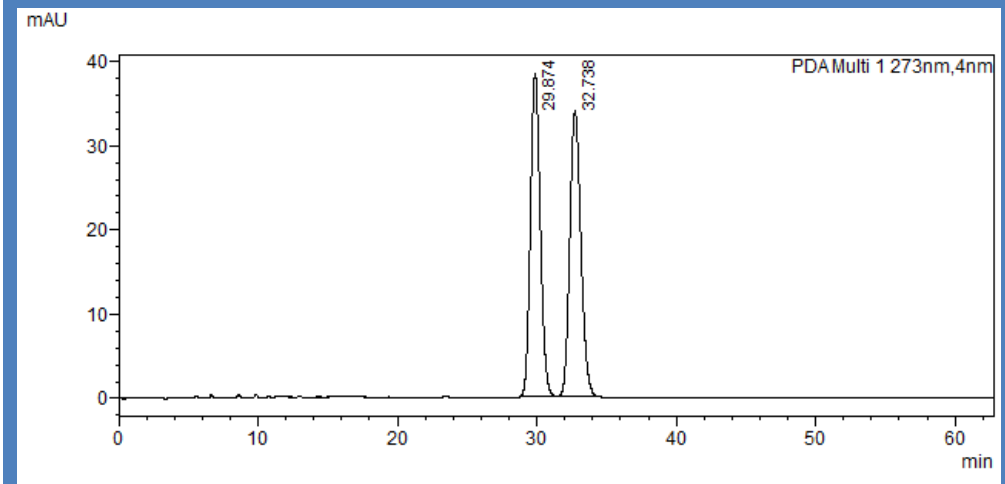
PDA Ch1 273nm

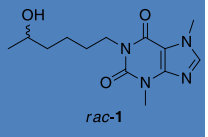
Peak#	Ret. Time	Area	Height	Height%	Area%
1	26.056	12869507	213797	51.770	49.995
2	28.875	12871965	199180	48.230	50.005
Total		25741472	412976	100.000	100.000

HPLC analytical separation for both enantiomers of *rac*-1 on Chiralpak AD-H at 25 °C

[Performed on a Shimadzu Nexera-*i* (LC-2040C 3D) equipped with a photodiode array detector (PAD)]

HPLC conditions: *n*-hexane-2-PrOH-DEA (78:22:0.1, v/v); f=1.0 mL/min; λ=273 nm; *p*=5.5 MPa





Peak Table

PDA Ch1 273nm

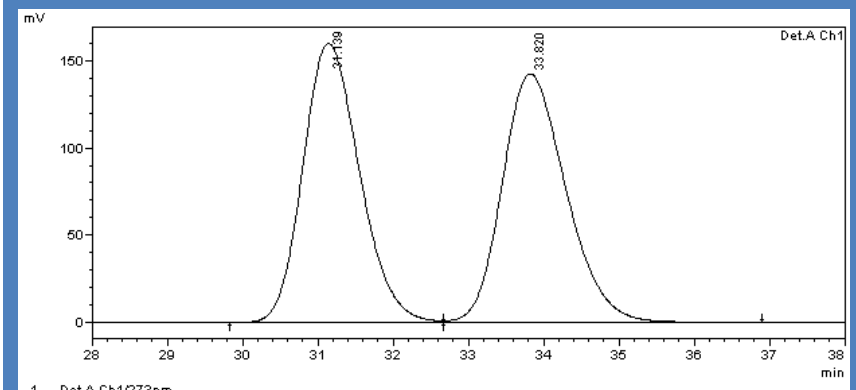
Peak#	Ret. Time	Area	Height	Height%	Area%
1	29.874	1880219	38309	53.061	50.086
2	32.738	1873753	33889	46.939	49.914
Total		3753971	72198	100.000	100.000

The HPLC analysis of whole microbial cells and ADHs-catalyzed stereoselective reductions of 3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione (2) – *Screening of the whole-cell biocatalysts*

HPLC analytical separation for both enantiomers of *rac*-1 on Chiralpak AD-H at 25 °C

[Performed on a Shimadzu Nexera-*i* (LC-2040C 3D) equipped with a photodiode array detector (PAD)]

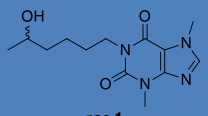
HPLC conditions: *n*-hexane-2-PrOH (78:22, v/v); f=1.0 mL/min; λ=273 nm; *p*=5.7 MPa



Peak Table

Detector A Ch1 273nm

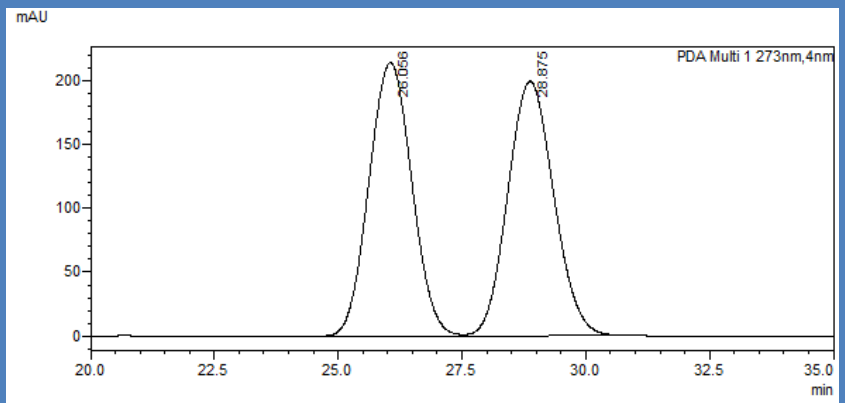
Peak#	Ret. Time	Area	Height	Area %	Height %
1	31.139	8366579	159713	49.912	52.890
2	33.820	8395948	142260	50.088	47.110
Total		16762527	301972	100.000	100.000



HPLC analytical separation for both enantiomers of *rac*-1 on Chiralpak AD-H at 25 °C

(Performed on a Shimadzu CTO-10ASV chromatograph equipped with STD-20A UV detector)

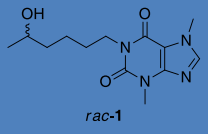
HPLC conditions: *n*-hexane-2-PrOH-DEA (78:22:0.1, v/v/v); f=1.0 mL/min; λ=273 nm; *p*=5.8 MPa



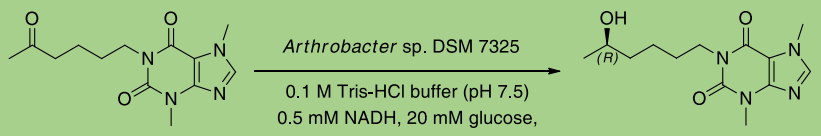
Peak Table

PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	26.056	12869507	213797	51.770	49.995
2	28.875	12871965	199180	48.230	50.005
Total		25741472	412976	100.000	100.000

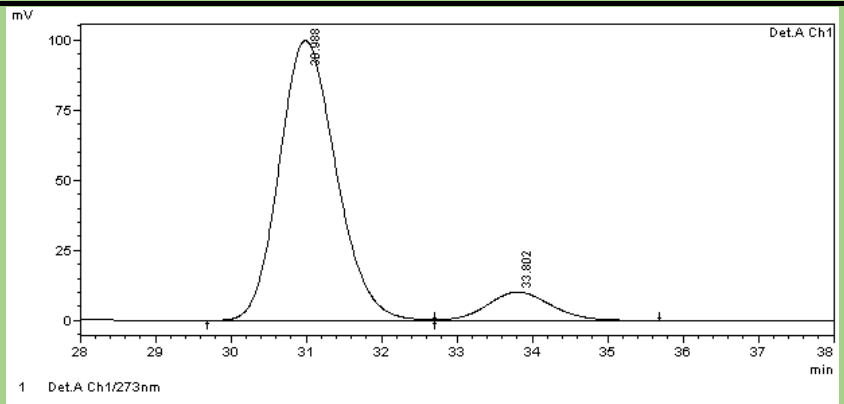


HPLC analysis for the subsequent biocatalytic reaction:



2

(*R*)-(–)-**1**
83% conv.,80% ee

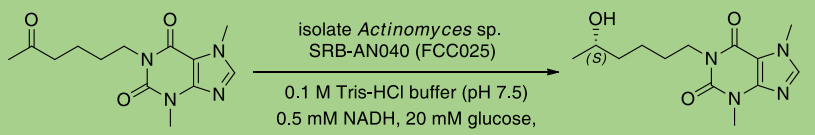


Peak Table

Detector A Ch1 273nm

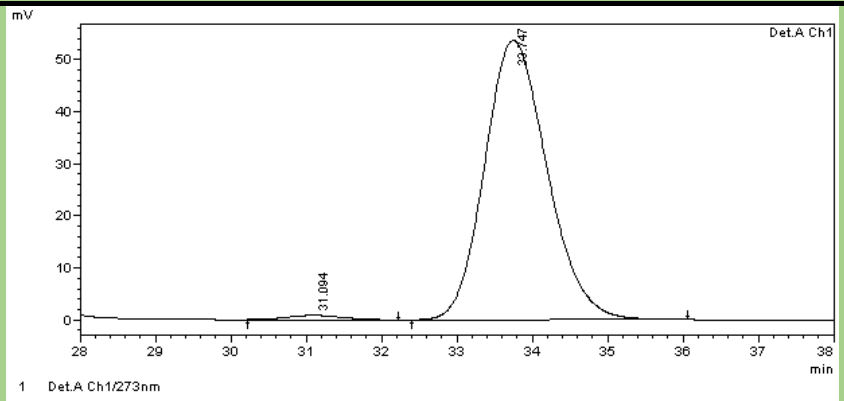
Peak#	Ret. Time	Area	Height	Area %	Height %
1	30.988	5231419	99911	89.746	90.781
2	33.802	597719	10146	10.254	9.219
Total		5829137	110057	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2

(*S*)-(+)-**1**
54% conv., 97% ee

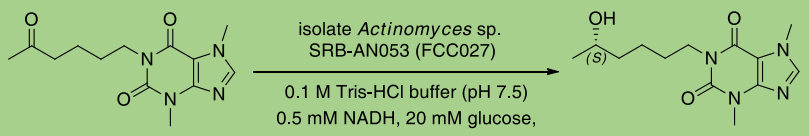


Peak Table

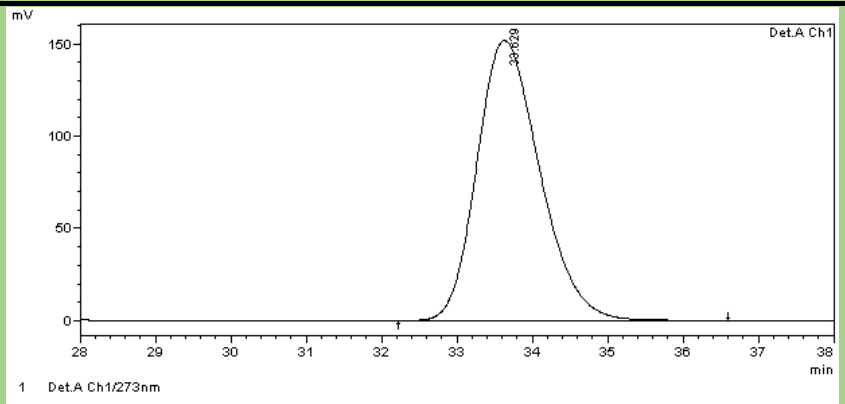
Detector A Ch1 273nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	31.094	41572	838	1.323	1.538
2	33.747	3101364	53675	98.677	98.462
Total		3142937	54513	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*S*)-(+)-**1**
92% conv., >99% ee

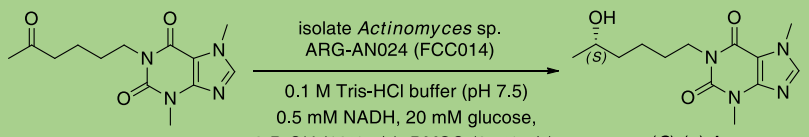


Peak Table

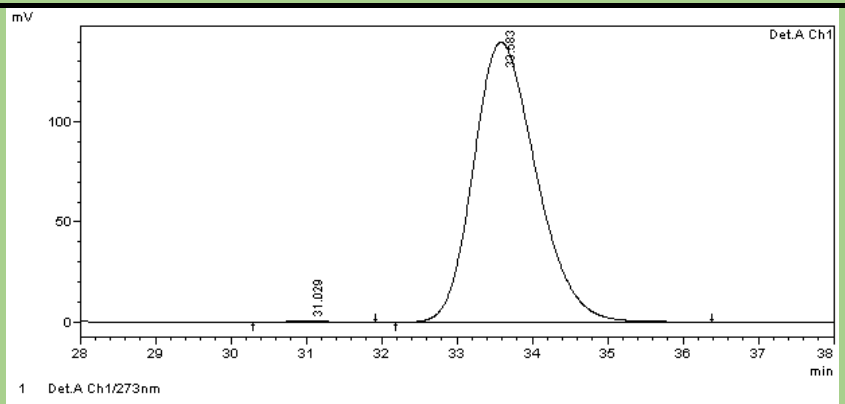
Detector A Ch1 273nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	33.629	8823860	151823	100.000	100.000
Total		8823860	151823	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*S*)-(+)-**1**
93% conv., >99% ee

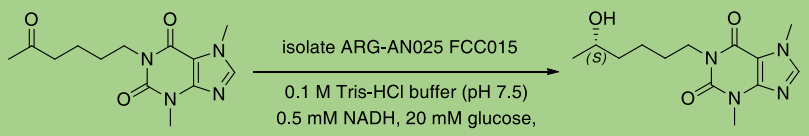


Peak Table

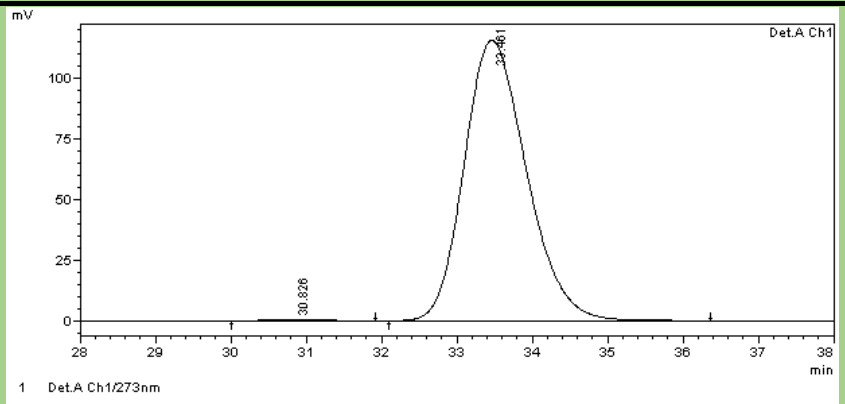
Detector A Ch1 273nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	31.029	15917	341	0.197	0.243
2	33.583	8079254	139621	99.803	99.757
Total		8095171	139962	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*S*)-(+)-**1**
94% conv., 99% ee

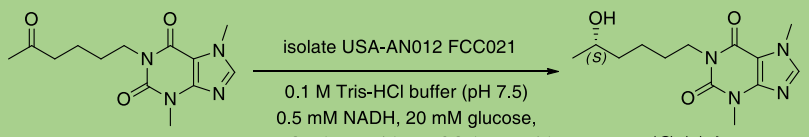


Peak Table

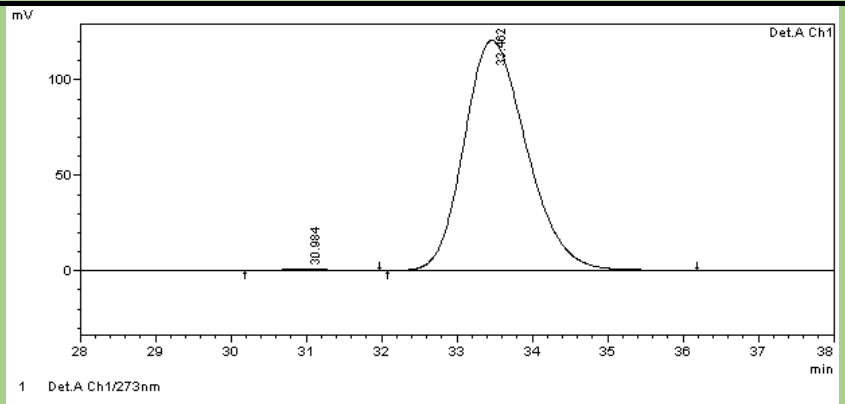
Detector A Ch1 273nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	30.826	24149	486	0.362	0.419
2	33.461	6653216	115506	99.638	99.581
Total		6677365	115992	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*S*)-(+)-**1**
92% conv., 99% ee

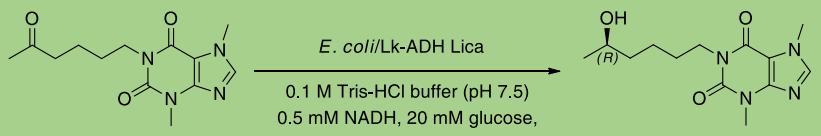


Peak Table

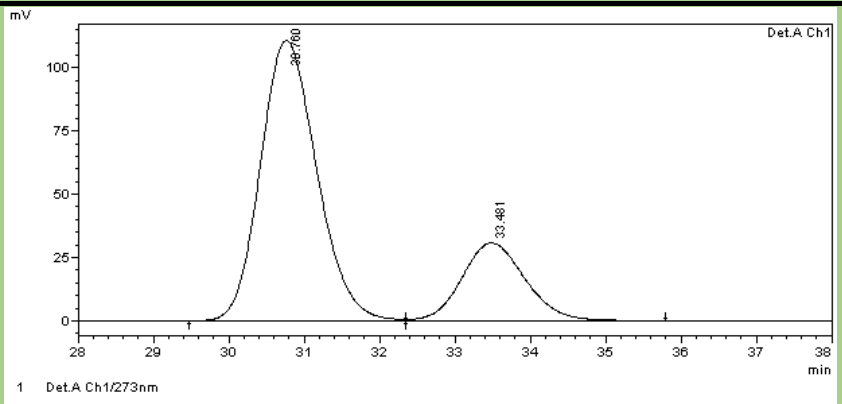
Detector A Ch1 273nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	30.984	22140	456	0.317	0.376
2	33.462	6953519	120585	99.683	99.624
Total		6975658	121041	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 (*R*)-(–)-**1**
98% conv., 53% ee

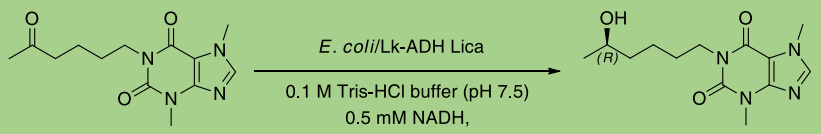


Peak Table

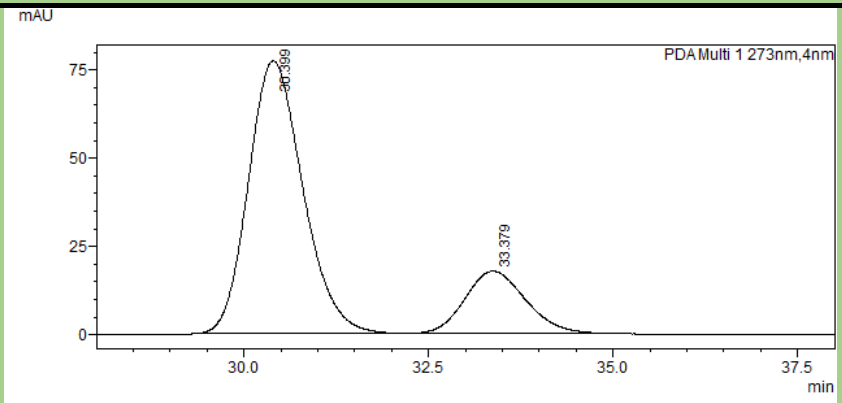
Detector A Ch1 273nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	30.760	5748370	110723	76.300	78.294
2	33.481	1785578	30697	23.700	21.706
Total		7533949	141420	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 (*R*)-(–)-**1**
97% conv., 60% ee

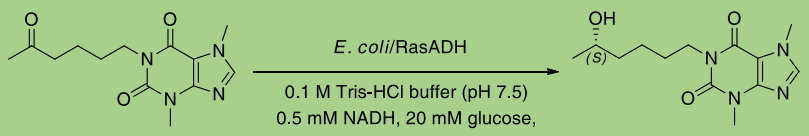


Peak Table

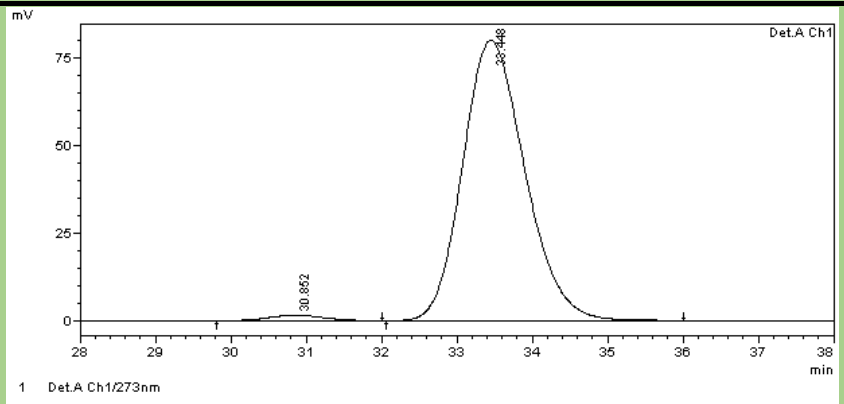
PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	30.399	3963866	77279	81.479	79.886
2	33.379	998028	17567	18.521	20.114
Total		4961893	94846	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*S*)-(+)-**1**
95% conv., 97% ee

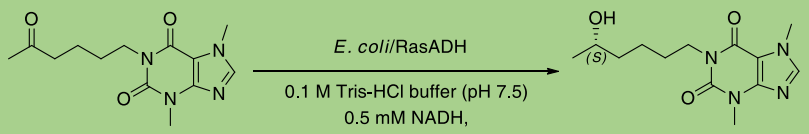


Peak Table

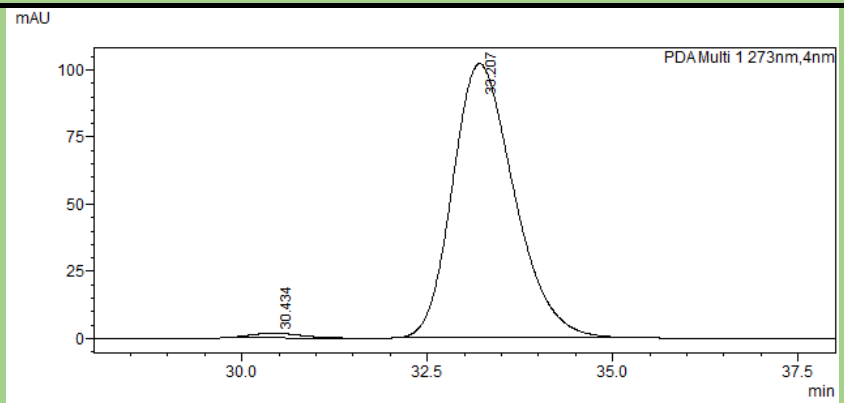
Detector A Ch1 273nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	30.852	78757	1559	1.688	1.918
2	33.448	4586236	79753	98.312	98.082
Total		4664992	81313	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*S*)-(+)-**1**
95% conv., 97% ee

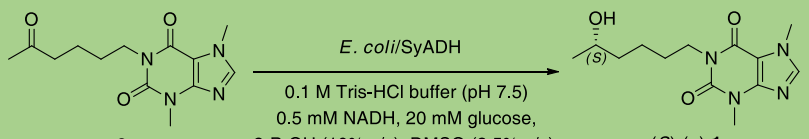


Peak Table

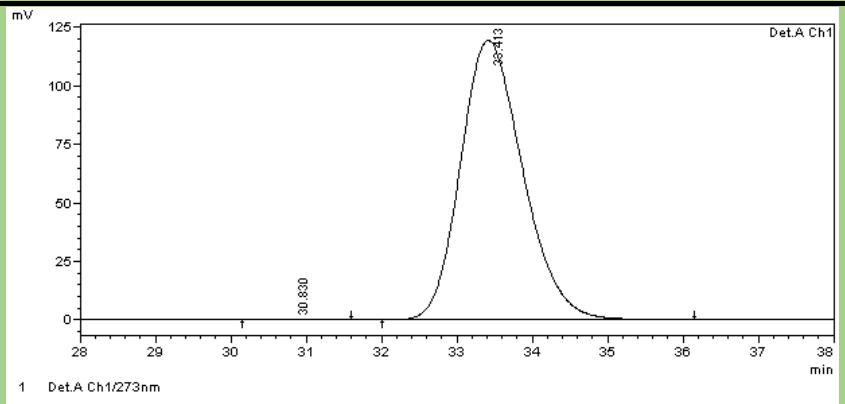
PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	30.434	84156	1804	1.738	1.409
2	33.207	5887106	101997	98.262	98.591
Total		5971262	103801	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*S*)-(+)-**1**
87% conv., >99% ee

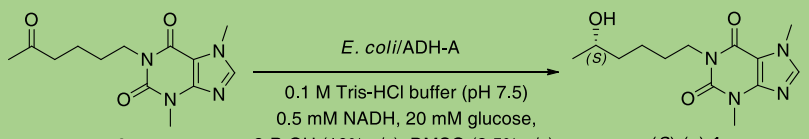


Peak Table

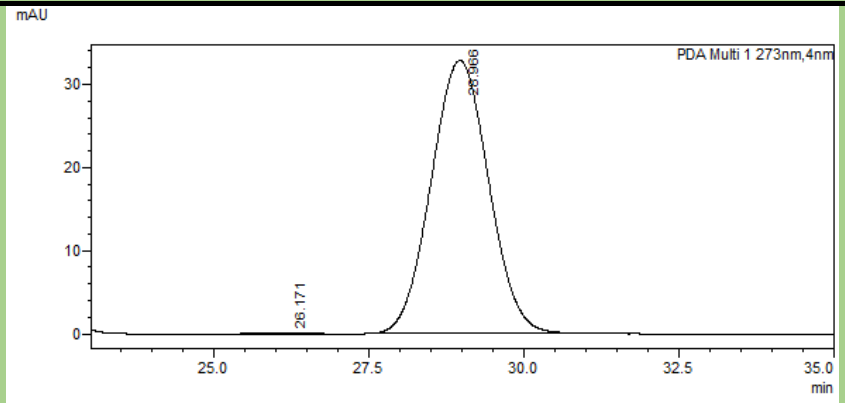
Detector A Ch1 273nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	30.830	17281	384	0.251	0.321
2	33.413	6859580	119395	99.749	99.679
Total		6876862	119779	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*S*)-(+)-**1**
98% conv., >99% ee

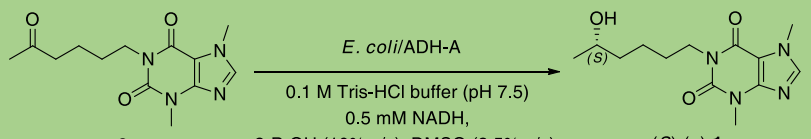


Peak Table

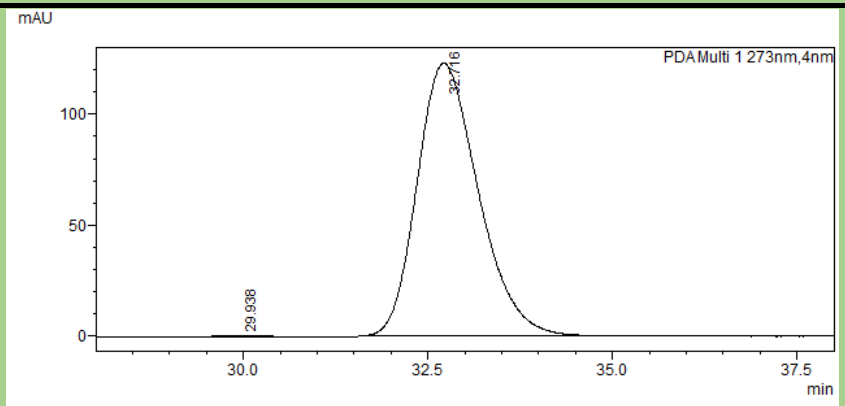
PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	26.171	4905	99	0.300	0.233
2	28.966	2102140	32703	99.700	99.767
Total		2107045	32801	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*S*)-(+)-**1**
98% conv., >99% ee

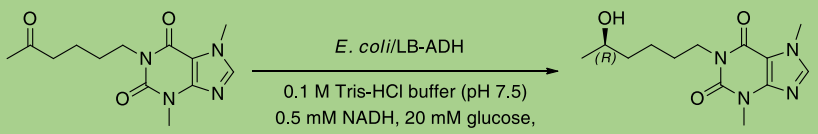


Peak Table

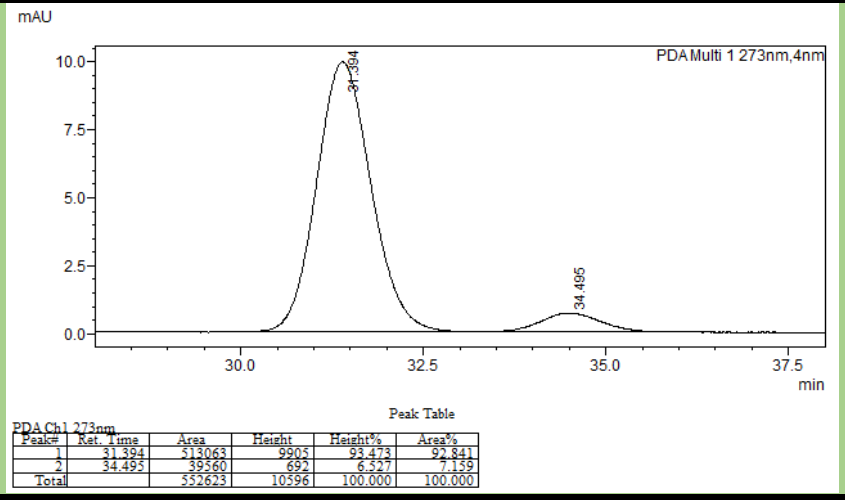
PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	29.938	9645	252	0.204	0.136
2	32.716	7098895	122928	99.796	99.864
Total		7108540	123179	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*R*)-(–)-**1**
41% conv., 86% ee

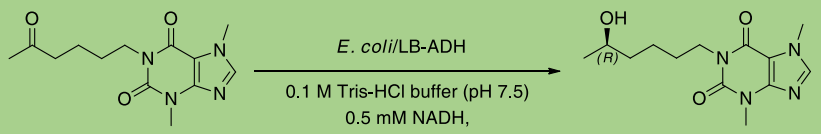


Peak Table

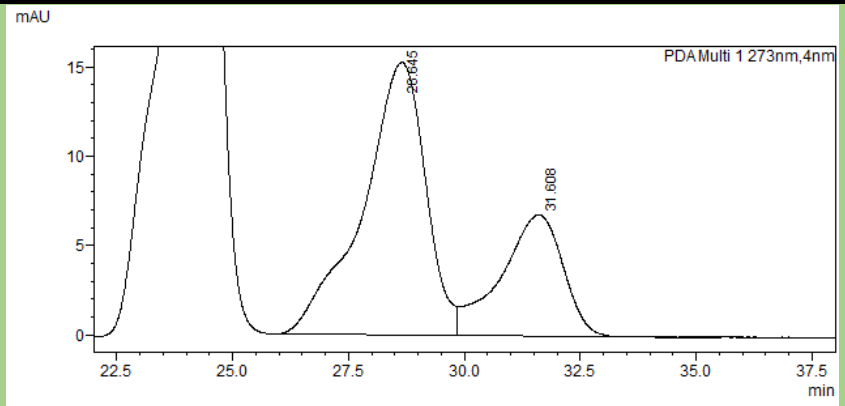
PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	31.394	513063	9905	93.473	92.841
2	34.495	39560	692	6.527	7.159
Total		552623	10596	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*R*)-(–)-**1**, 37% conv., 39% ee

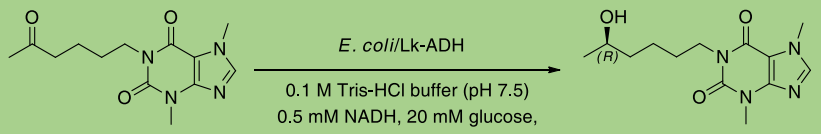


Peak Table

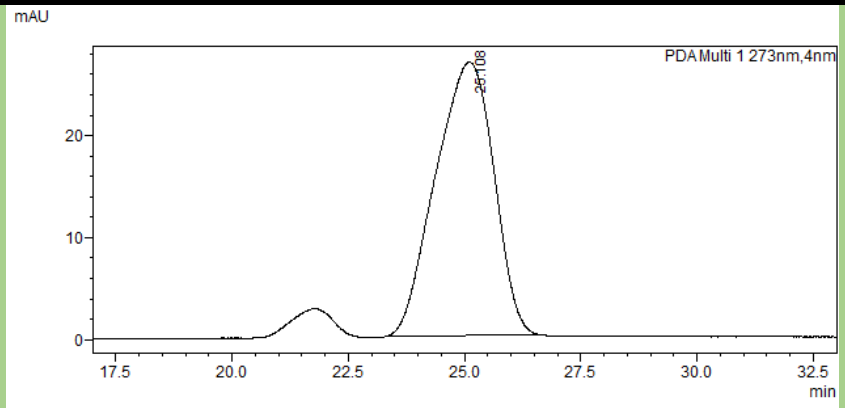
PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	28.645	1433525	15258	69.245	69.453
2	31.608	630486	6777	30.755	30.547
Total		2064011	22034	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*R*)-(–)-**1**, 96% conv., >99% ee

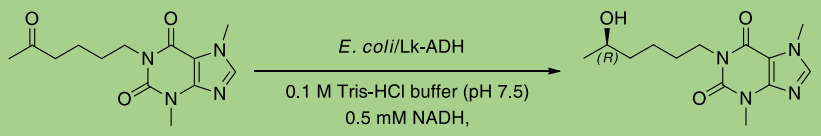


Peak Table

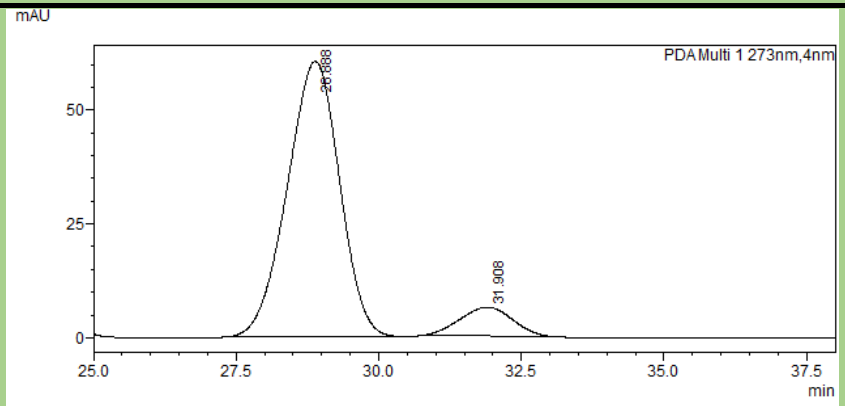
PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	25.108	2337867	26774	100.000	100.000
Total		2337867	26774	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*R*)-(–)-**1**
97% conv., 81% ee

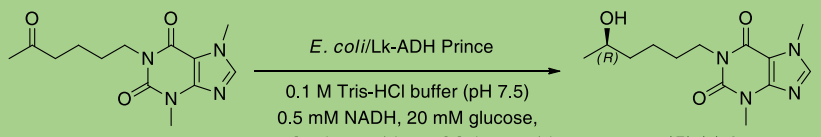


Peak Table

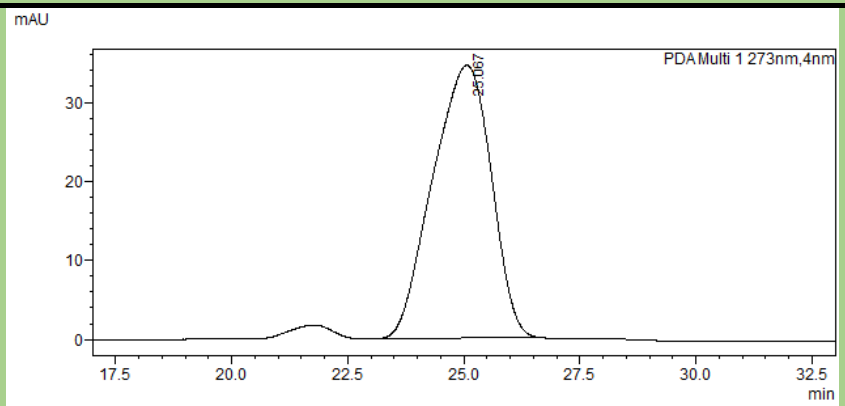
PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	28.888	3853056	60443	90.437	90.255
2	31.908	416039	6391	9.563	9.745
Total		4269095	66834	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*R*)-(–)-**1**
98% conv., >99% ee

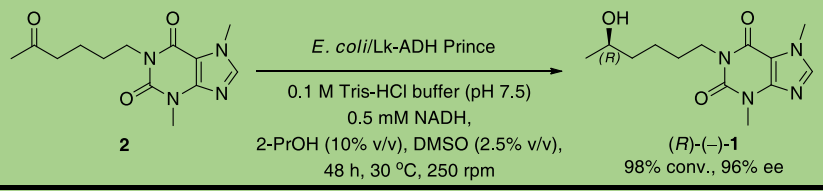


Peak Table

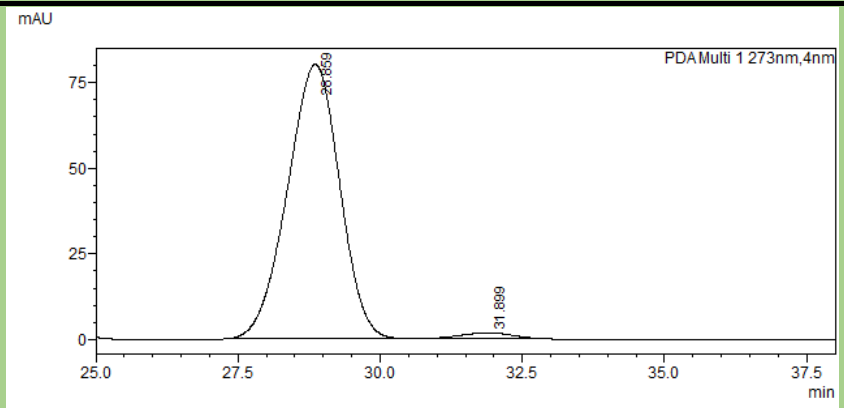
PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	25.067	3028212	34515	100.000	100.000
Total		3028212	34515	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*R*)-(–)-**1**, 98% conv., 96% ee



Peak Table

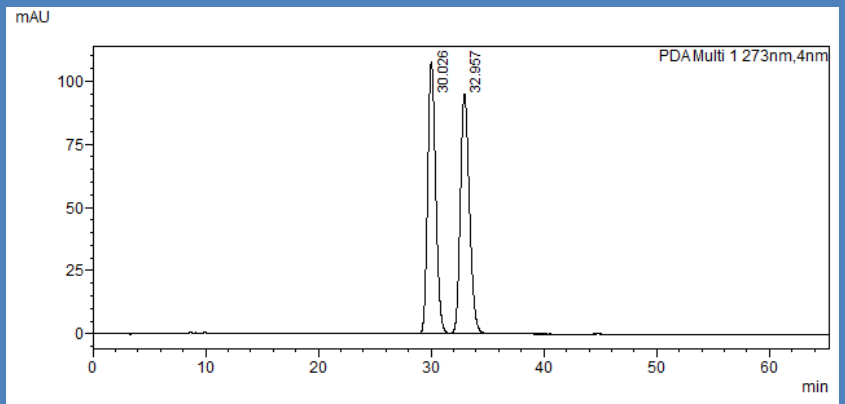
PDA Ch1 273nm

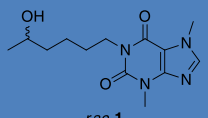
Peak#	Ret. Time	Area	Height	Height%	Area%
1	28.859	5123082	80071	97.920	97.999
2	31.899	104631	1701	2.080	2.001
Total		5227713	81772	100.000	100.000

The HPLC analysis of ADHs-catalyzed stereoselective reductions of 3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione (2) – *Up-scaling*

HPLC analytical separation for both enantiomers of *rac*-1 on Chiralpak AD-H at 25 °C

HPLC conditions: *n*-hexane-2-PrOH (78:22, v/v); f=1.0 mL/min; λ=273 nm; *p*=5.7 MPa





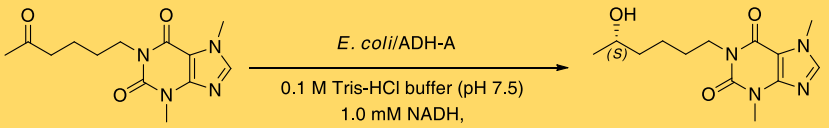
rac-1

Peak Table

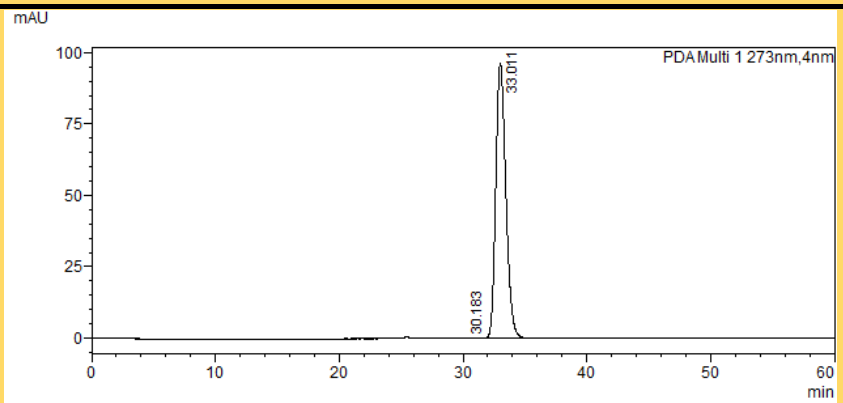
PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	30.026	5288793	107318	53.200	50.068
2	32.957	5274527	94406	46.800	49.932
Total		10563320	201723	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*S*)-(+)-**1**
93% conv., 67% yield, >99% ee

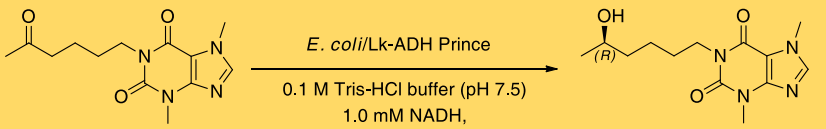


Peak Table

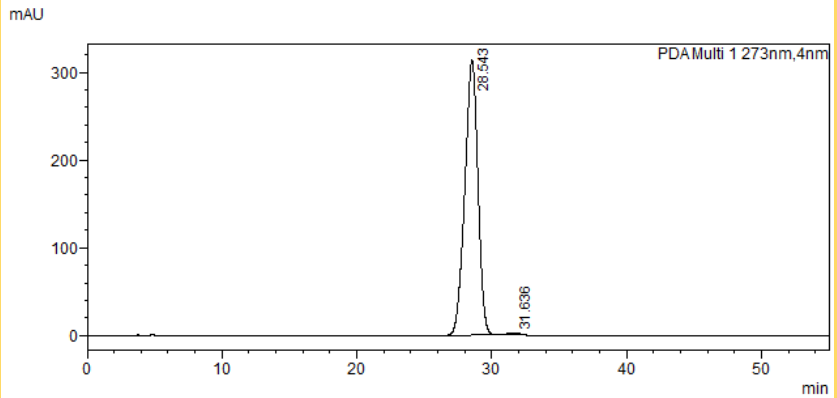
PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	30.183	6728	193	0.200	0.124
2	33.011	5422286	96126	99.800	99.876
Total		5429013	96319	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 → (*R*)-(–)-**1**
94% conv., 65% yield, >99% ee

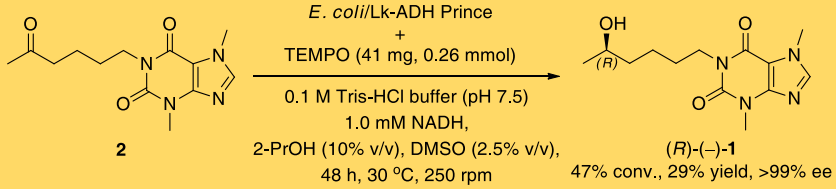


Peak Table

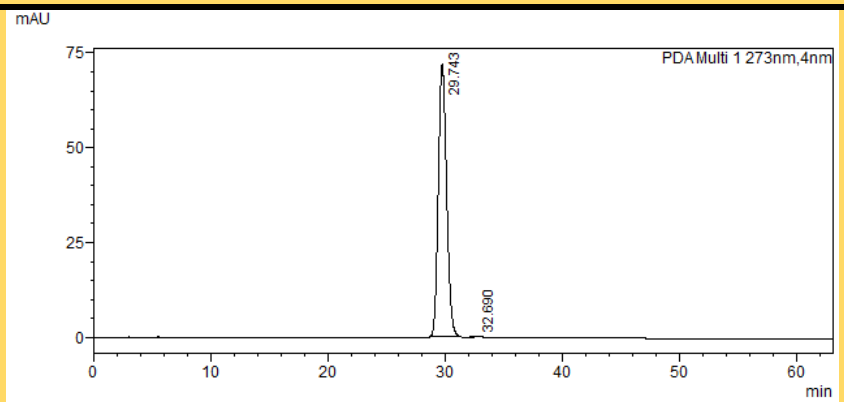
PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	28.543	21223107	313510	99.386	99.549
2	31.636	96153	1938	0.614	0.451
Total		21319260	315447	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



2 (*R*)-(–)-**1**
47% conv., 29% yield, >99% ee



Peak Table

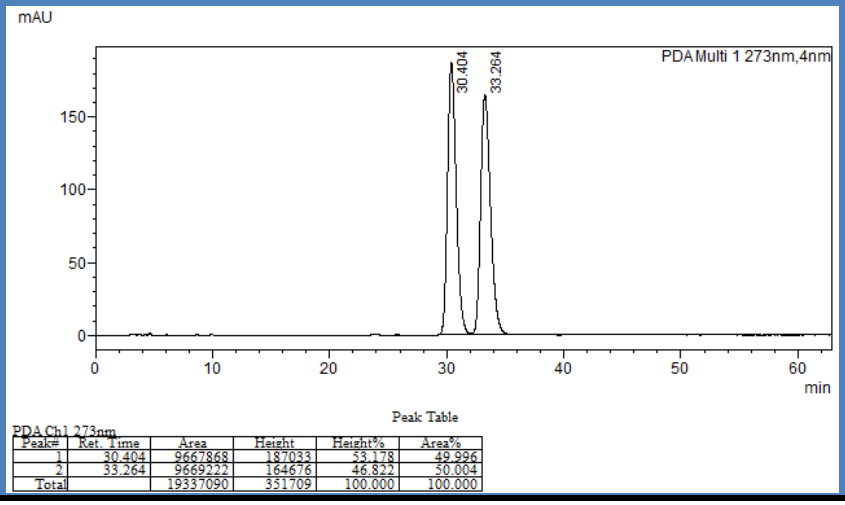
PDA Ch1 273nm

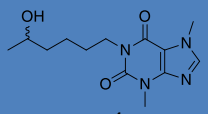
Peak#	Ret. Time	Area	Height	Height%	Area%
1	29.743	3535754	71886	99.575	99.673
2	32.690	11587	307	0.425	0.327
Total		3547340	72193	100.000	100.000

The HPLC analysis of semi-preparative (laccase/TEMPO)-ADH-catalyzed deracemization of lisofylline (*rac*-1)

HPLC analytical separation for both enantiomers of *rac*-1 on Chiralpak AD-H at 25 °C

HPLC conditions: *n*-hexane-2-PrOH (78:22, v/v); f=1.0 mL/min; λ=273 nm; *p*=5.7 MPa





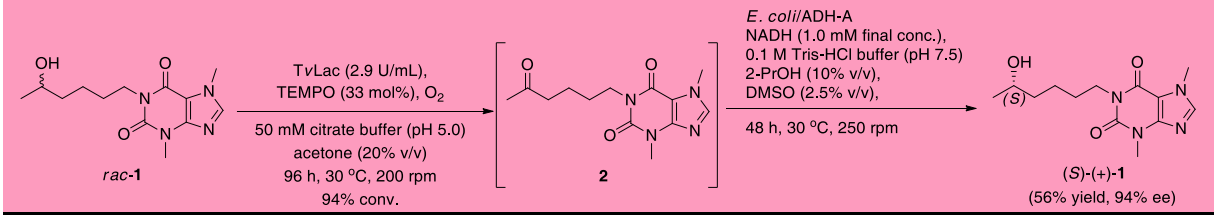
rac-1

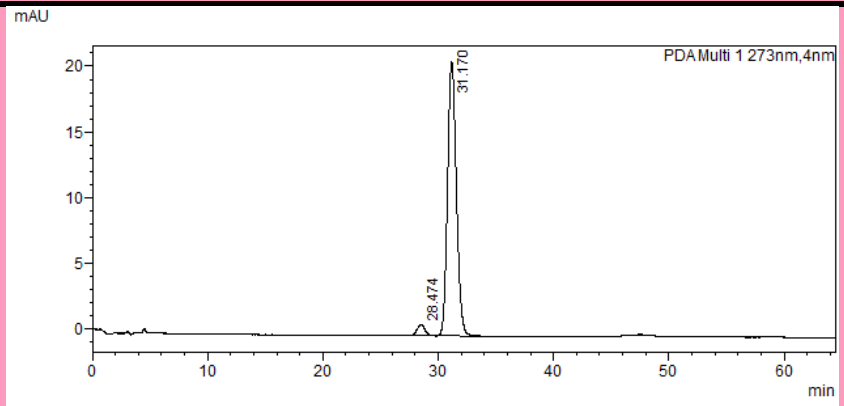
Peak Table

PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	30.404	9667868	187033	53.178	49.996
2	33.264	9669222	164676	46.822	50.004
Total		19337090	351709	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



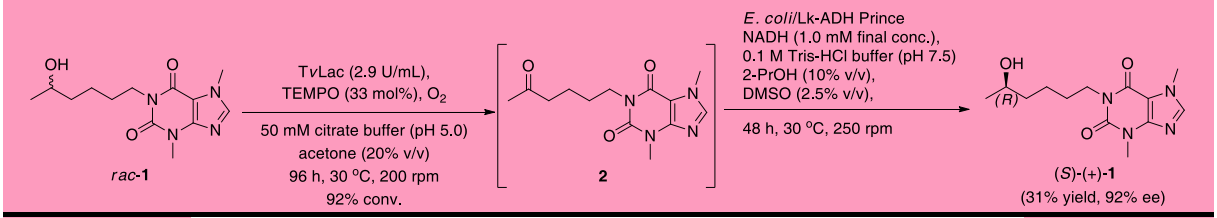


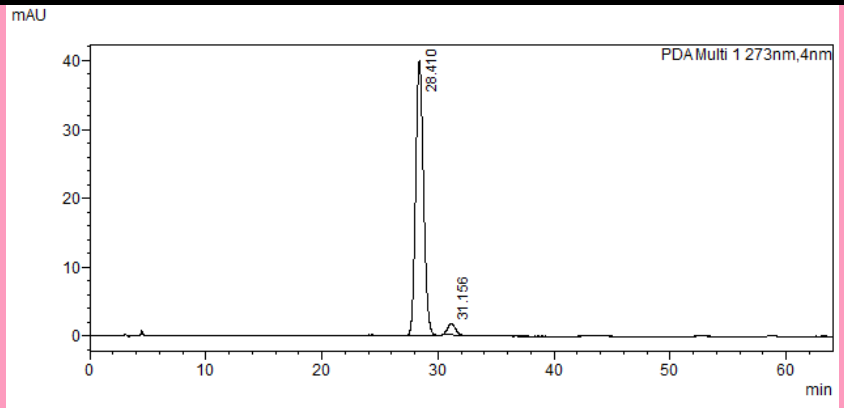
Peak Table

PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	28.474	36059	814	3.761	3.154
2	31.170	1107308	20830	96.239	96.846
Total		1143367	21644	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:



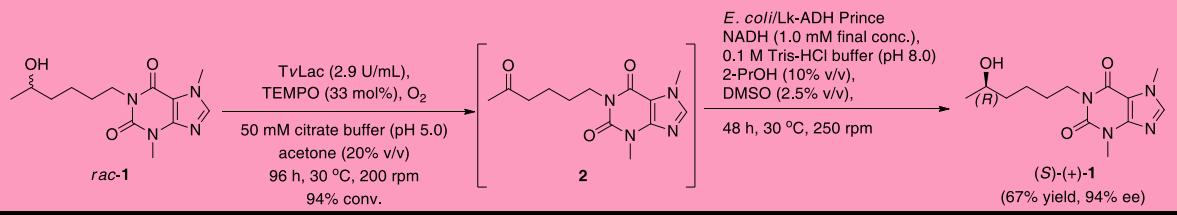


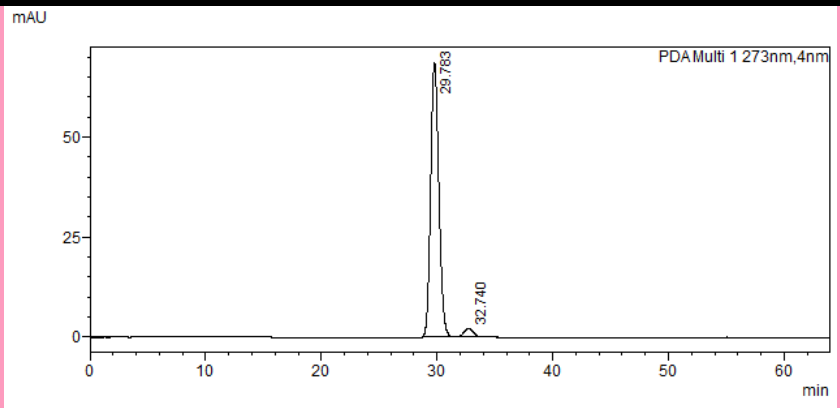
Peak Table

PDA Ch1 273nm

Peak#	Ret. Time	Area	Height	Height%	Area%
1	28.410	1873314	39843	96.170	96.166
2	31.156	74681	1587	3.830	3.834
Total		1947995	41430	100.000	100.000

HPLC analysis for the subsequent biocatalytic reaction:





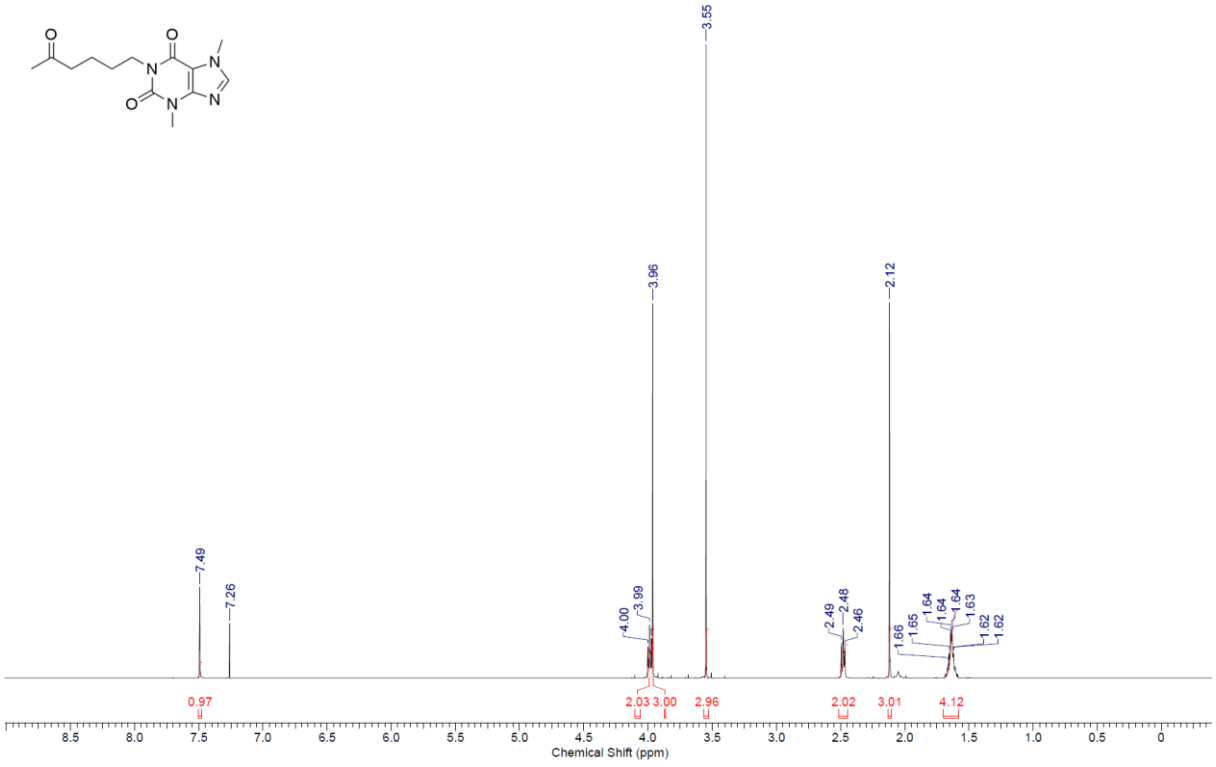
Peak Table

PDA Ch1 273nm

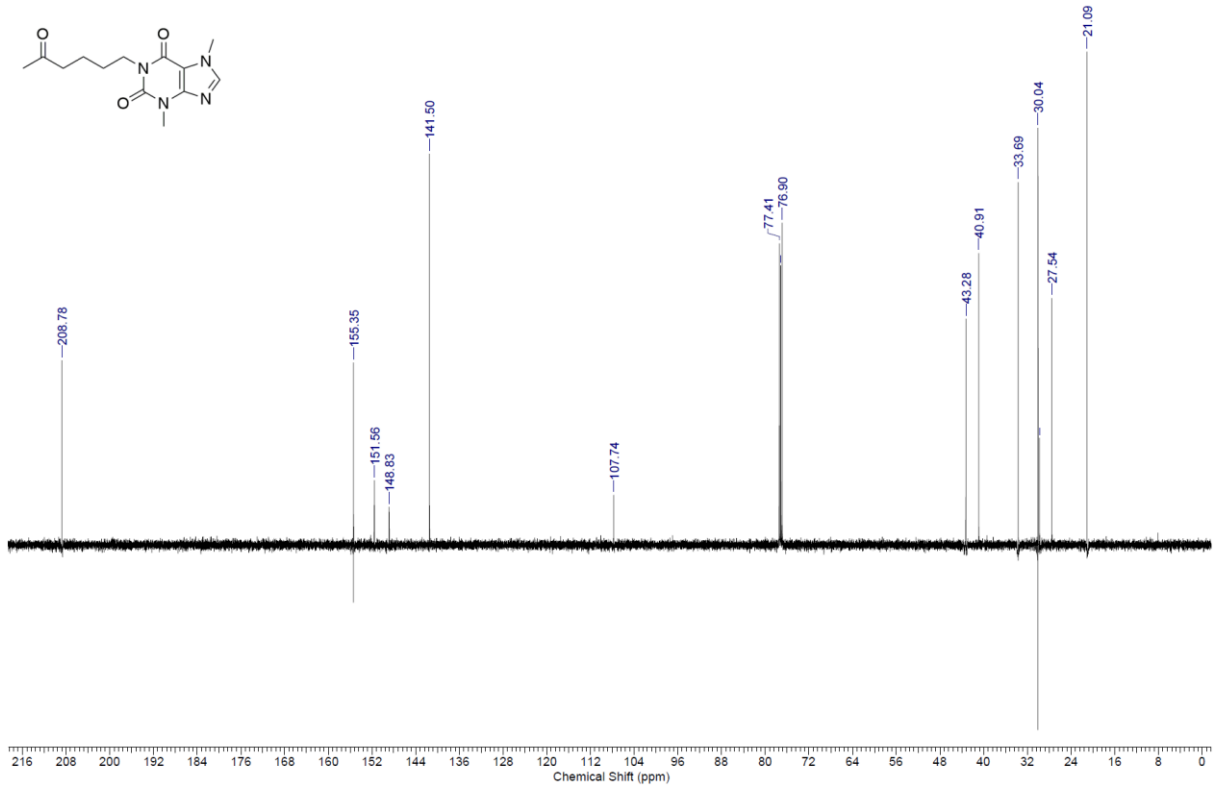
Peak#	Ret. Time	Area	Height	Height%	Area%
1	29.783	3374059	68429	97.070	96.948
2	32.740	106214	2065	2.930	3.052
Total		3480273	70495	100.000	100.000

3,7-Dimethyl-1-(5-oxohexyl)-3,7-dihydro-1H-purine-2,6-dione (2)

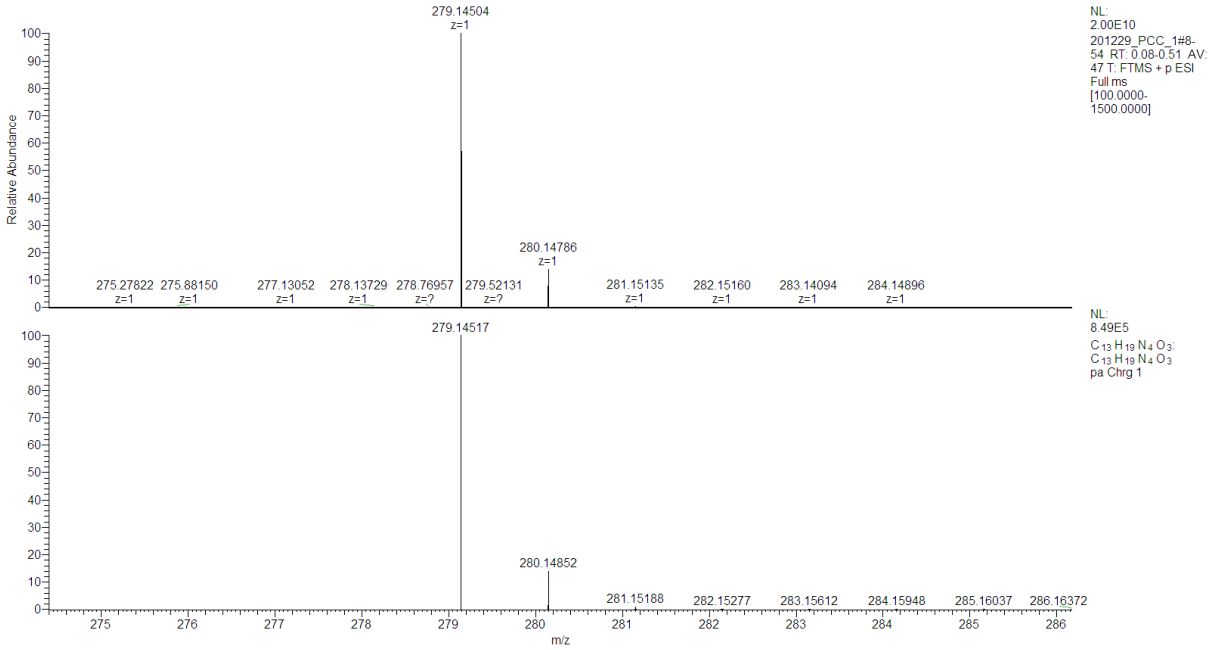
^{1}H NMR spectrum of **2** (500 MHz, $CDCl_3$)



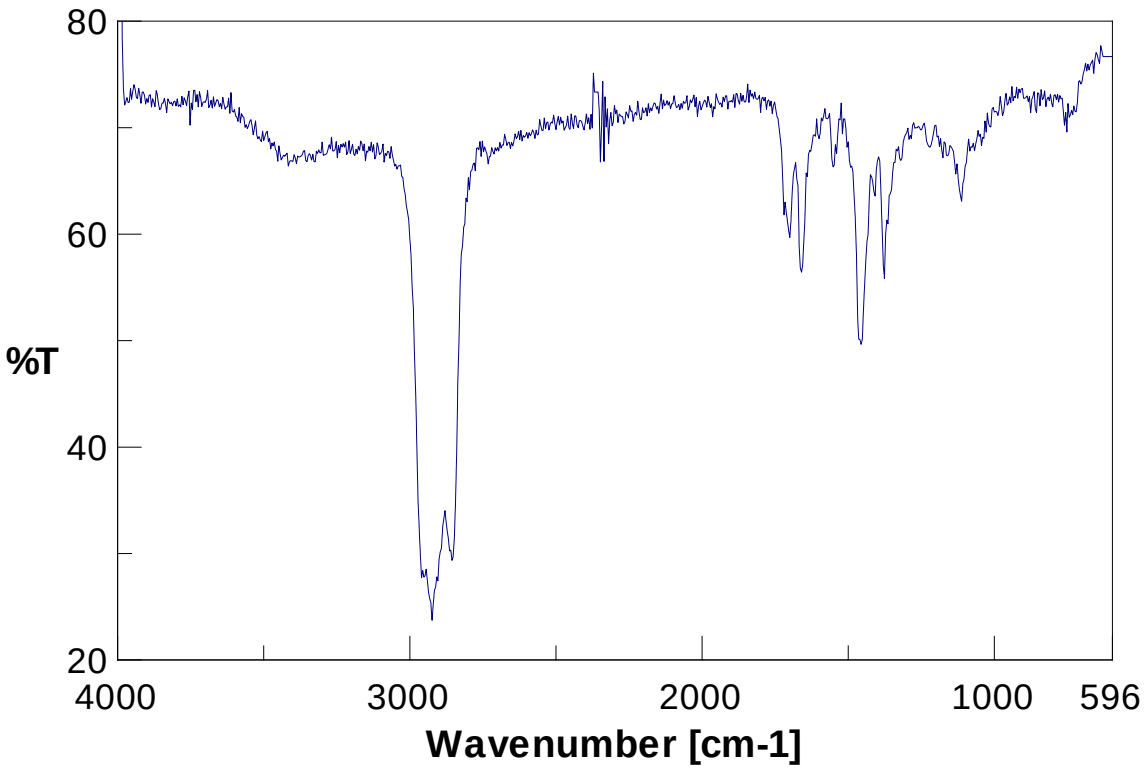
^{13}C NMR spectrum of **2** (126 MHz, $CDCl_3$)



FTMS spectrum of **2** (ESI-TOF)

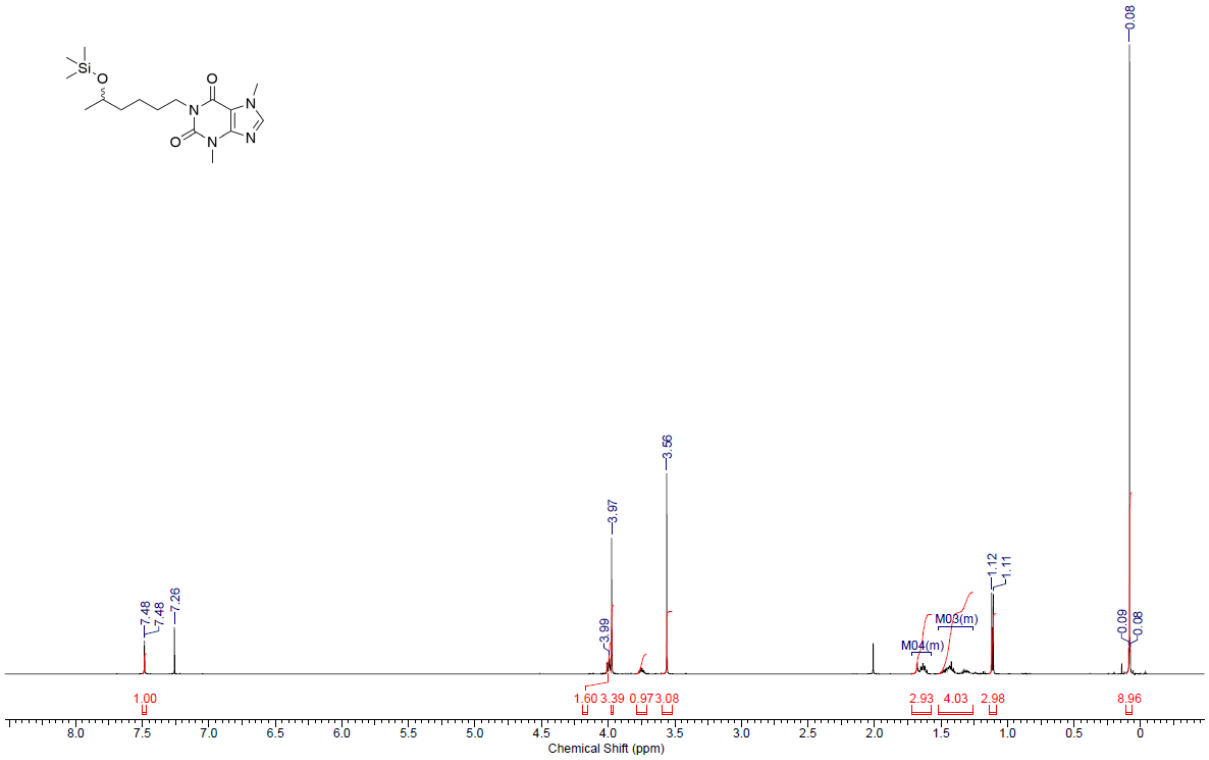


IR spectrum of **2** (Mineral oil, Nujol)

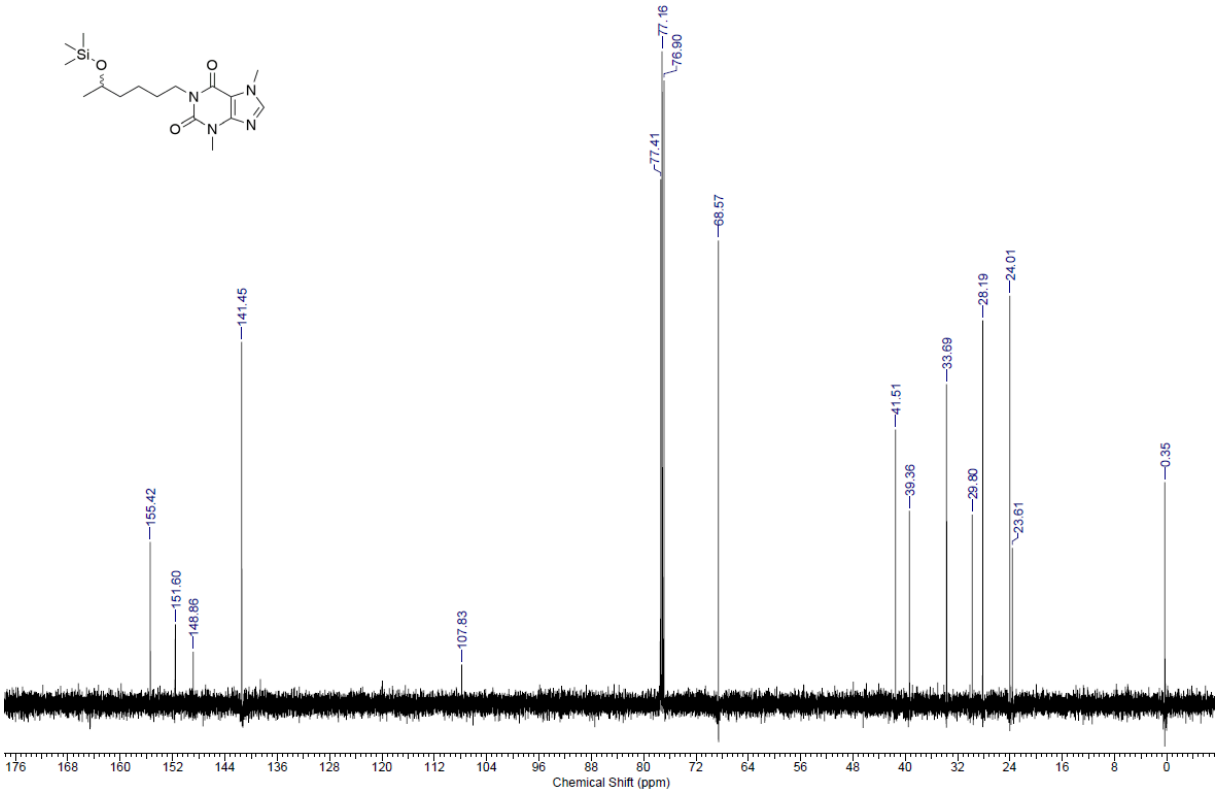


3,7-Dimethyl-1-{5-[(trimethylsilyl)oxy]hexyl}-2,3,6,7-tetrahydro-1H-purine-2,6-dione (rac-3)

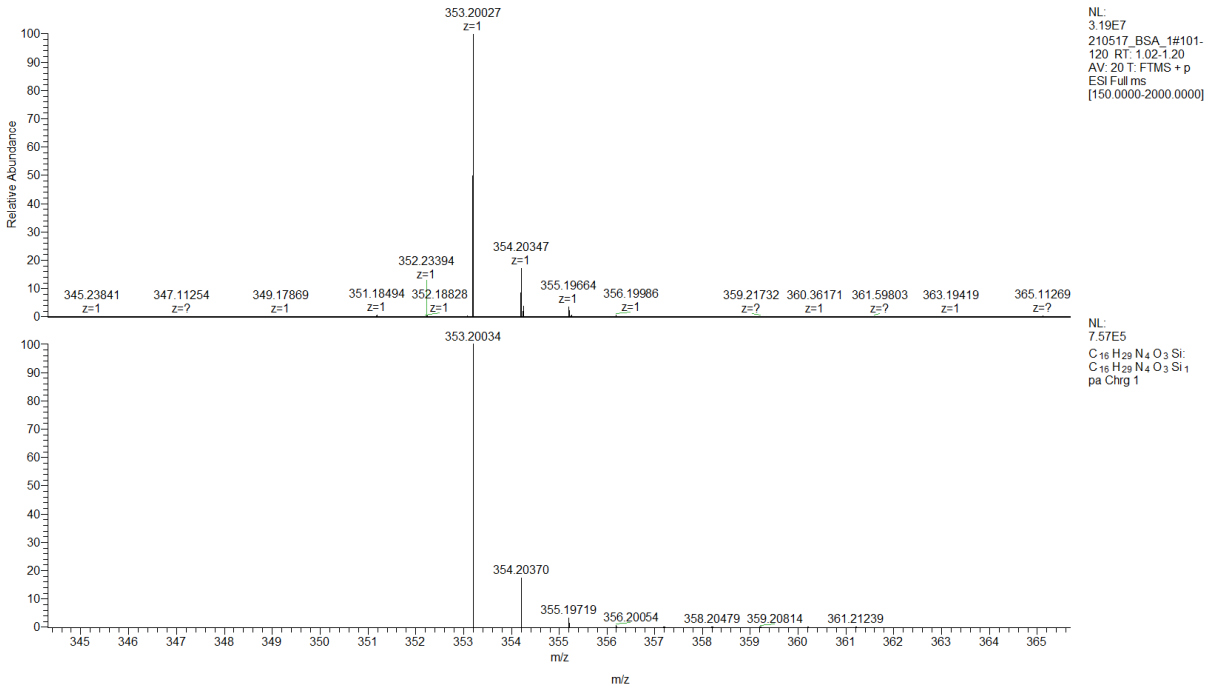
^{1}H NMR spectrum of *rac*-**3** (500 MHz, $CDCl_3$)



^{13}C NMR spectrum of *rac*-**3** (126 MHz, $CDCl_3$)



FTMS spectrum of *rac*-**3** (ESI-TOF)



IR spectrum of *rac*-**3** (Mineral oil, Nujol)