Supporting Information

Engineering a Thermostable Transketolase for Arylated Substrates

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Table S1 Kinetic and enantioselectivity data for the best hits of TK_{gst}

L382X/D470X TK_{gst} variant	3-Phenyl-propanal (U/mg)[a]	(*S*)-1,3-dihydroxy-5-phenylpentan-2-one ee (%)[b]
w.t.	0.01±0.009	22±0.7
Leu/Thr	0.06±0.029	75±2.7
Ser/Glu	0.08±0.006	26±0.7
Asn/Val	0.08±0.004	67±3.5
Asn/Glu	0.09±0.019	73±3.5
Val/Leu	0.1±0.017	80±1.0
Leu/Ile	0.11±0.026	65±0.0
Met/Val	0.15±0.009	71±3.5
Asn/Ser	0.16±0.006	81±0.4
Glu/Leu	0.16±0.011	54±2.6
Met/Ser	0.16±0.002	76±0.5
Ser/Ser	0.18±0.005	32±4.2
Asp/Ser	0.18±0.019	61±1.4
Met/Thr	0.18±0.006	80±0.6
Ala/Ile	0.19±0.021	04±2.5
Phe/Ser	0.19±0.015	99±0.1
Met/Leu	0.28±0.008	43±2.9

[a] Assay solution (200 µL) contained TK (25 µg), LiHP (50 mM), 3-phenylpropanal (10 mM), ThDP (2.4 mM), $MgCl_2$ (9 mM), phenol red (0.028 mM), DMSO (20 % v/v) and triethanolamine buffer (2 mM, pH 7.5); [b] ee was determined by HPLC using Chiralpak IB column with Hexane & isopropanol (95:05) as mobile phase at 1mL/min flow rate

Table S2. Kinetic data for the best TK_{gst} variants towards aryl-containing aldehydes

	Specific Activity (U/mg)[a]					
Acceptor scope	**Wild-type**	**L382M/D470L**	**L382M/D470T**	**L382M/D470S**	**L382N/D470S**	**L382F/D470S**
Phenyl acetaldehyde	0.01±0.0002	0.20±0.005	0.19±0.000	0.12±0.001	0.17±0.004	0.19±0.010
3-Phenylpropanal	0.01±0.009	0.28±0.008	0.18±0.006	0.16±0.002	0.16±0.006	0.19±0.015
Phenoxy acetaldehyde	0.00±0.001	0.11±0.005	0.11±0.003	0.08±0.005	0.12±0.006	0.09±0.002
Benzyloxyacetaldehyde	0.02±0.001	0.11±0.002	0.09±0.008	0.08±0.004	0.09±0.008	0.09±0.009
3-[(Cbz)amino]propanal	0.01±0.004	0.18±0.005	0.09±0.010	0.08±0.005	0.13±0.004	0.11±0.007

[a] Assay solution (200 µL) contained TK (25 µg), LiHP (50 mM), acceptor aldehhydes (10 mM), ThDP (2.4 mM), $MgCl_2$ (9 mM), phenol red (0.028 mM), DMSO (20 % v/v) and triethanolamine buffer (2 mM, pH 7.5)

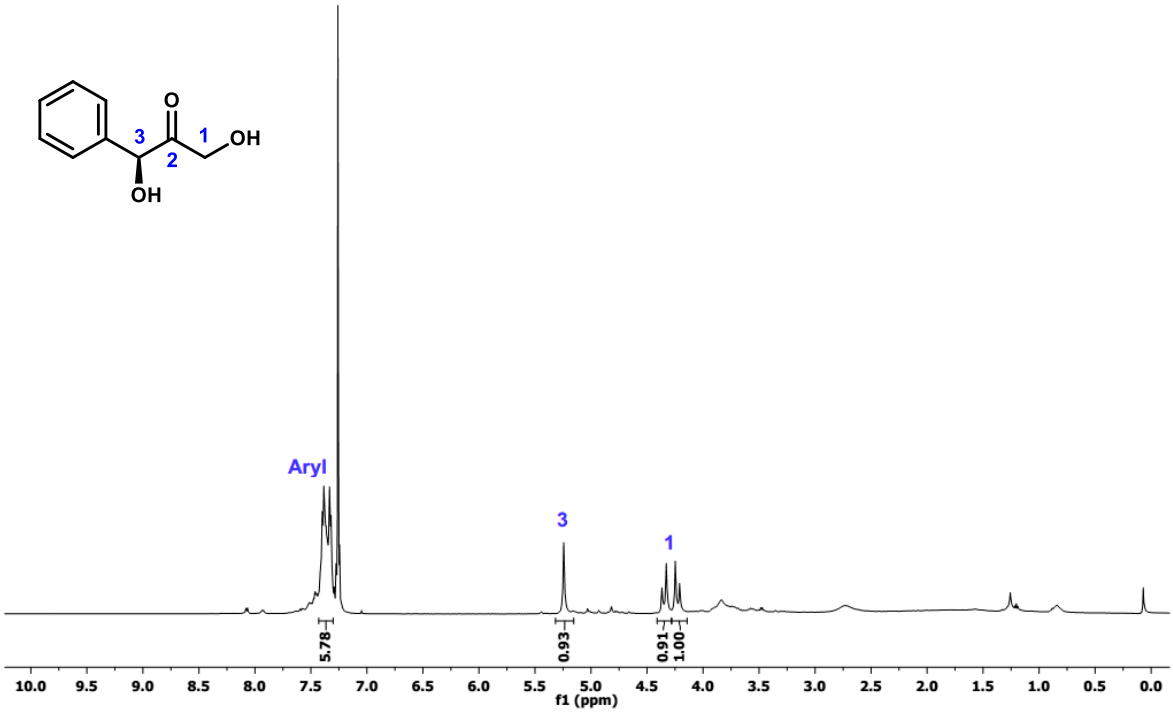
General procedure for *ee* determination by chiral shift experiments

(*S*)-2,2,2-Trifluoro-1-(9-anthryl)ethanol (TFAE; Sigma-Aldrich) was used as chiral solvating agent to determine *ee* values of TK products. ^{1}H NMR data were recorded for samples dissolved in $CDCl_3$ at room temperature on Bruker AR-300 spectrometer. Spectra were recorded first without additive, then TFAE was added and a second set of spectra was recorded.
Nonequivalence of protons at C1 was evident for *rac*-**2c** in the presence of 3 eq of (*S*)-TFAE (S11). Enantiomeric purity of substrates **2b – 4** was determined using 2 eq of (*S*)-TFAE.

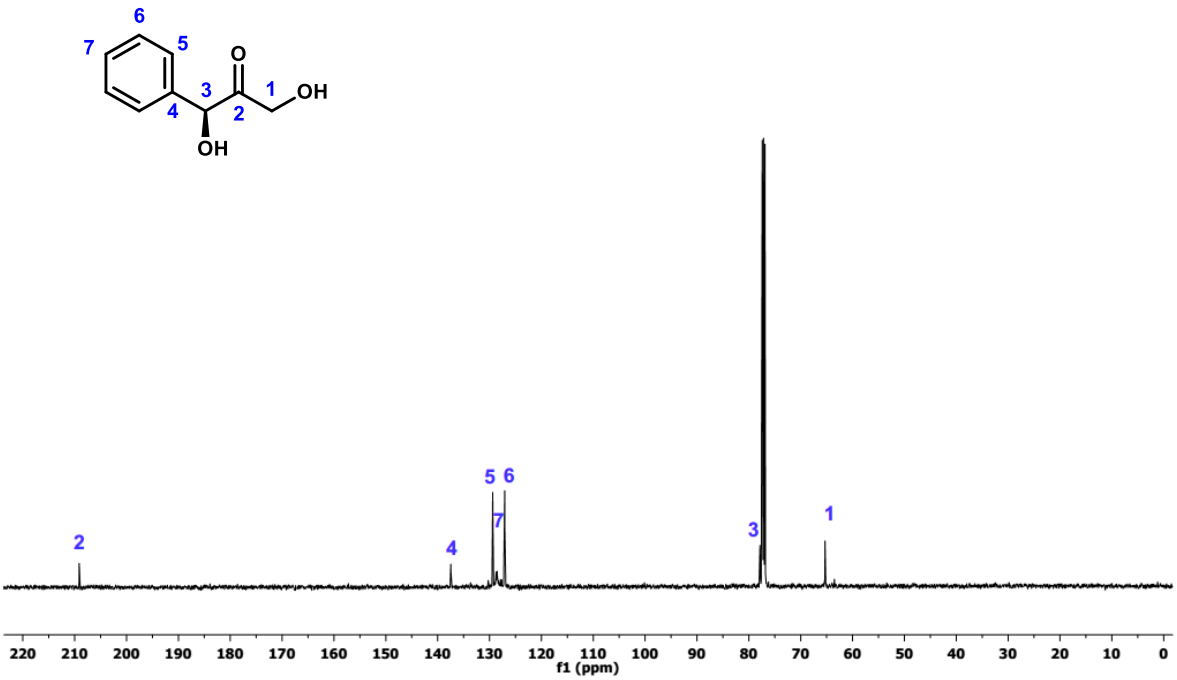
Table S3. Absolute concentrations of compounds **2b – 4** and (*S*)-TFAE

compound	**conc.$_{compound}$** [mmol / mL]	**conc.$_{TFAE}$** [mmol / mL]	***ee***
2b	0,14	0,26	>98%
2c	0,13	0,26	>98%
3	0,04	0,09	>98%
4	0,07	0,14	>98%

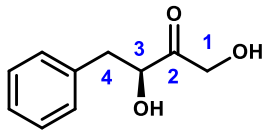
NMR Spectra

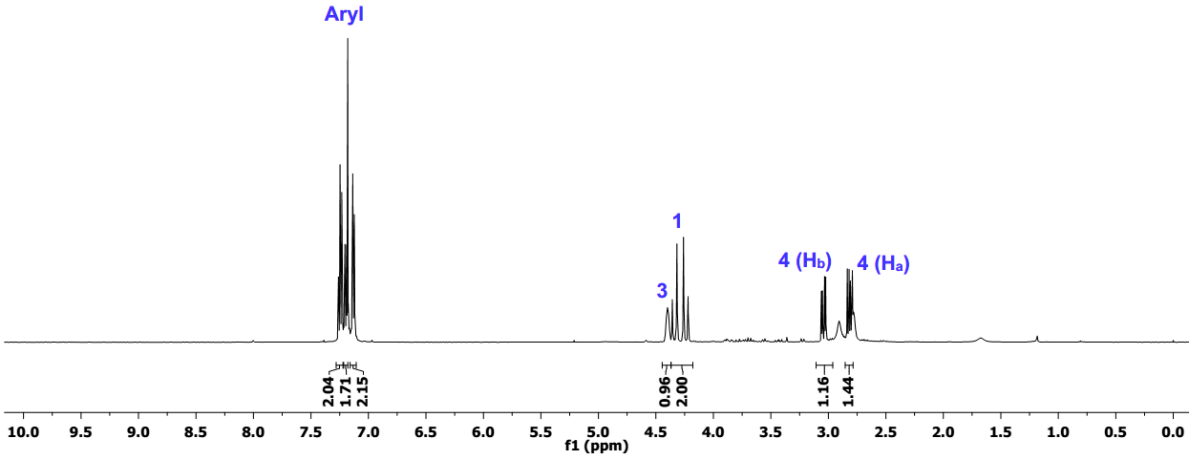


^{1}H NMR spectrum of (*R*)-1,3-dihydroxy-1-phenylpropan-2-one **(2a)** (500 MHz, $CDCl_3$)

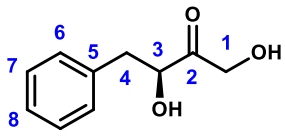


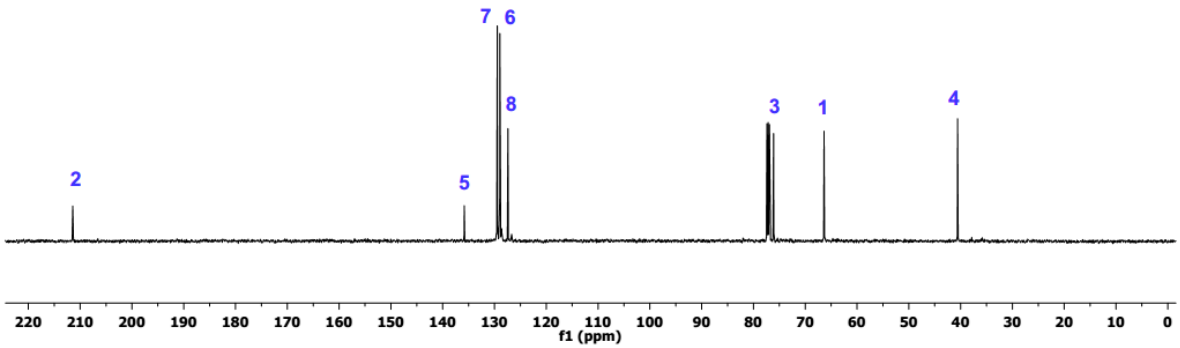
^{13}C NMR spectrum of (*R*)-1,3-dihydroxy-1-phenylpropan-2-one **(2a)** (125 MHz, $CDCl_3$)



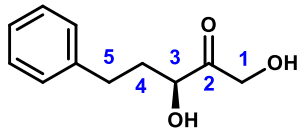


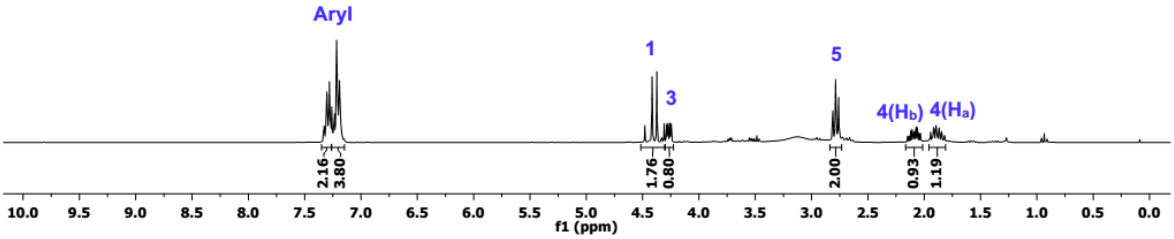
^{1}H NMR spectrum of (*S*)-1,3-dihydroxy-4-phenylbutan-2-one **(2b)** (500 MHz, $CDCl_3$)





^{13}C NMR spectrum of (*S*)-1,3-dihydroxy-4-phenylbutan-2-one **(2b)** (125 MHz, $CDCl_3$)





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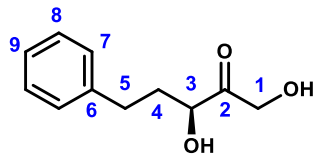

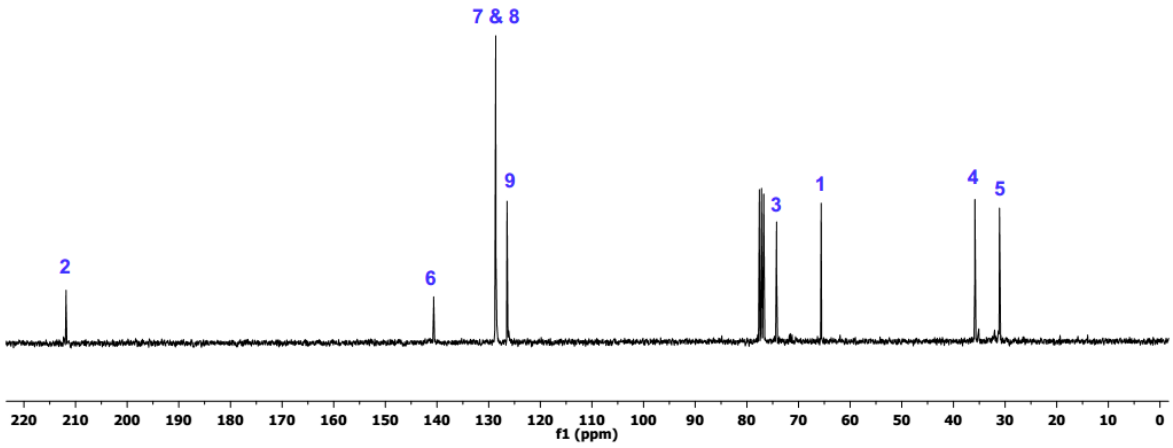

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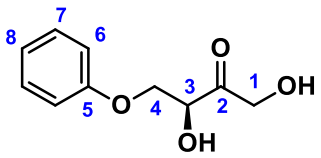
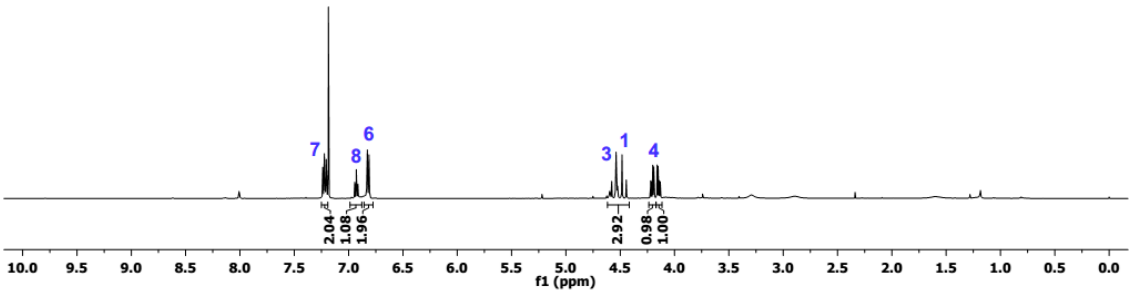
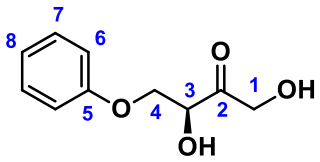
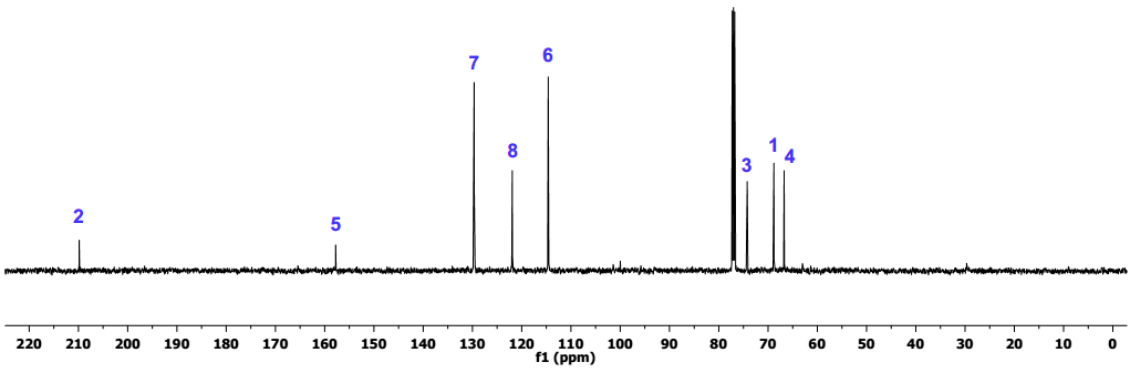


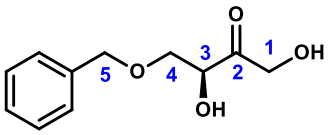


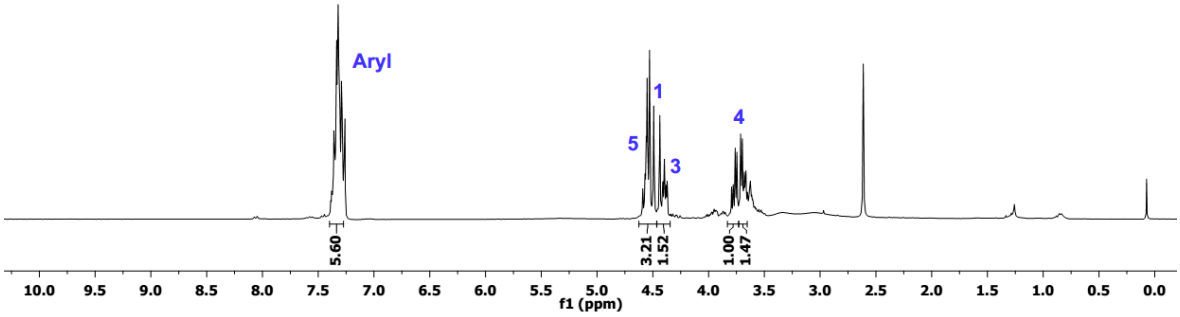
^{1}H NMR spectrum of (*S*)-1,3-dihydroxy-4-phenoxybutan-2-one **(3)** (500 MHz, $CDCl_3$)



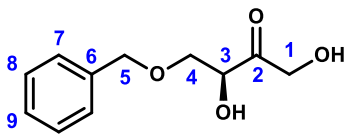


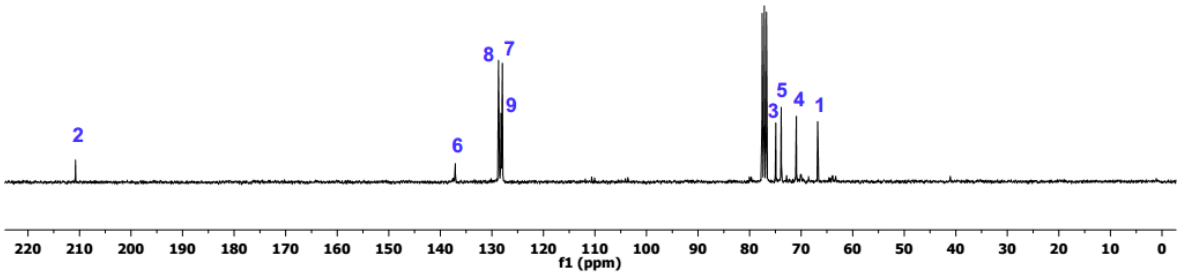
^{13}C NMR spectrum of (*S*)-1,3-dihydroxy-4-phenoxybutan-2-one **(3)** (125 MHz, $CDCl_3$)



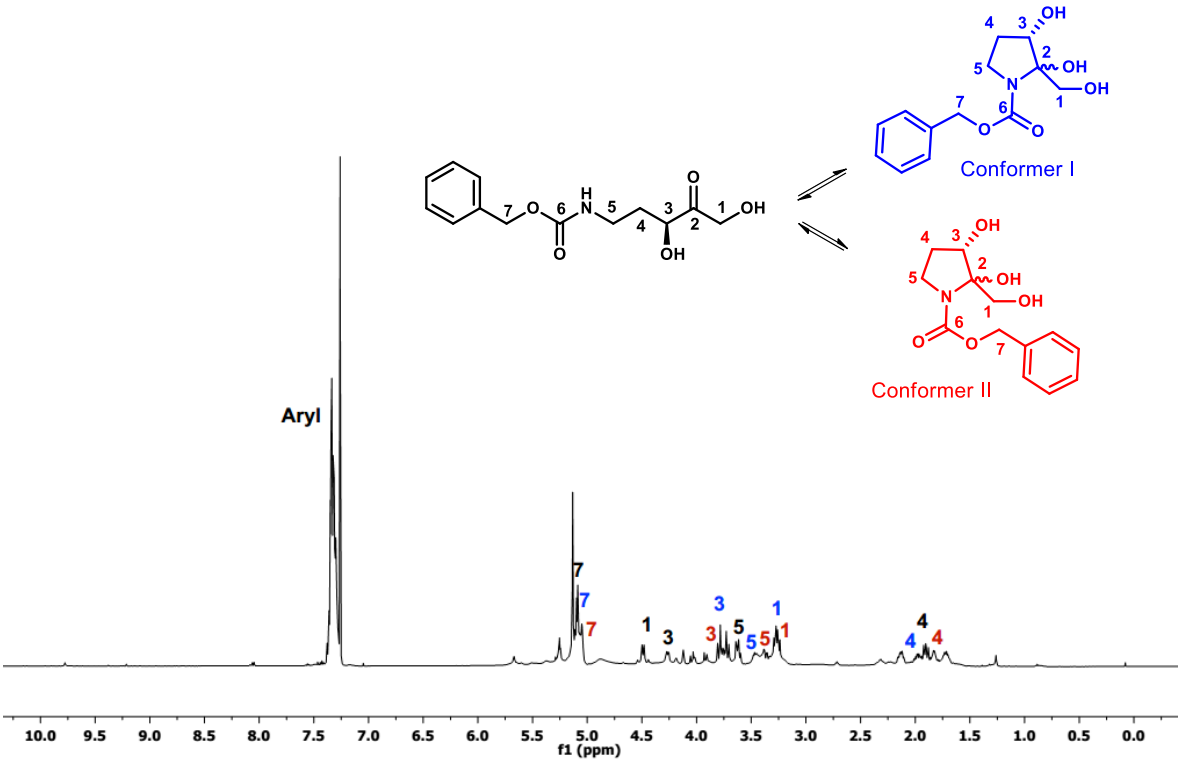


^{1}H NMR spectrum of (*S*)-4-(benzyloxy)-1,3-dihydroxybutan-2-one **(4)** (300 MHz, $CDCl_3$)

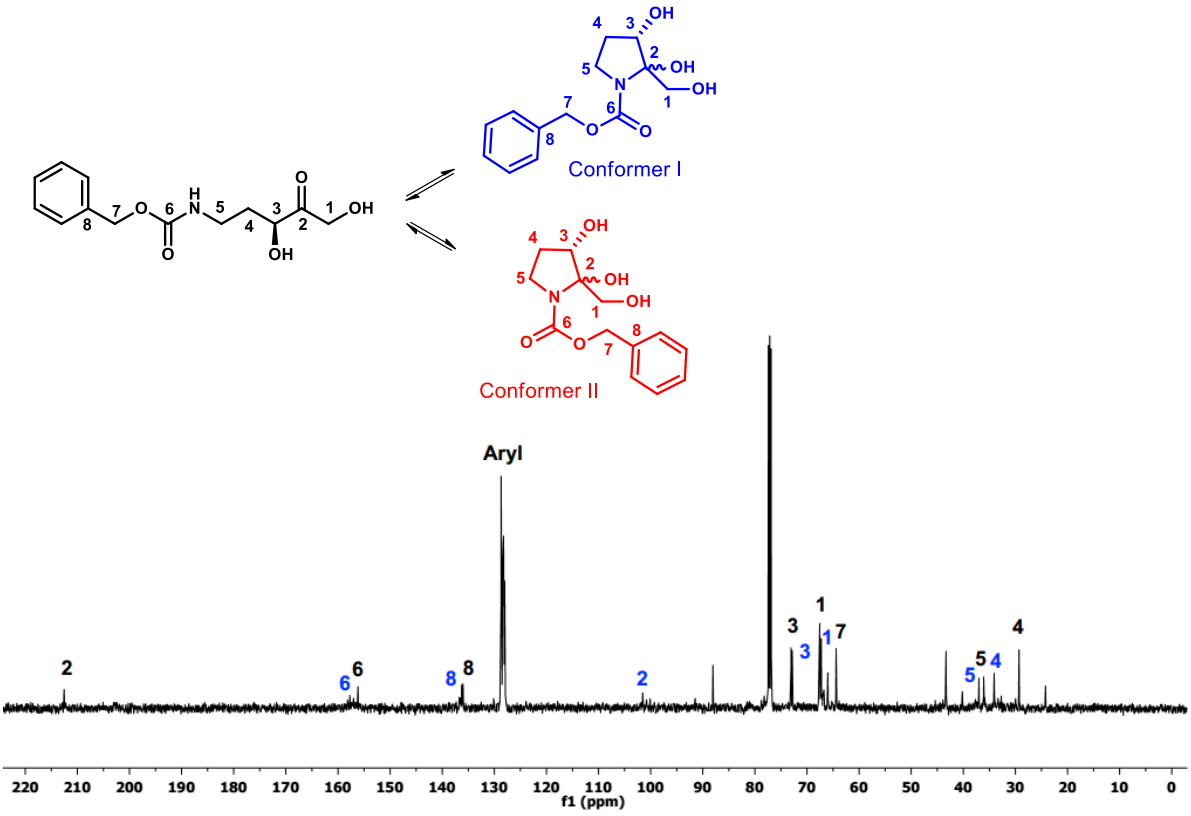




^{13}C NMR spectrum of (*S*)-4-(benzyloxy)-1,3-dihydroxybutan-2-one **(4)** (75 MHz, $CDCl_3$)



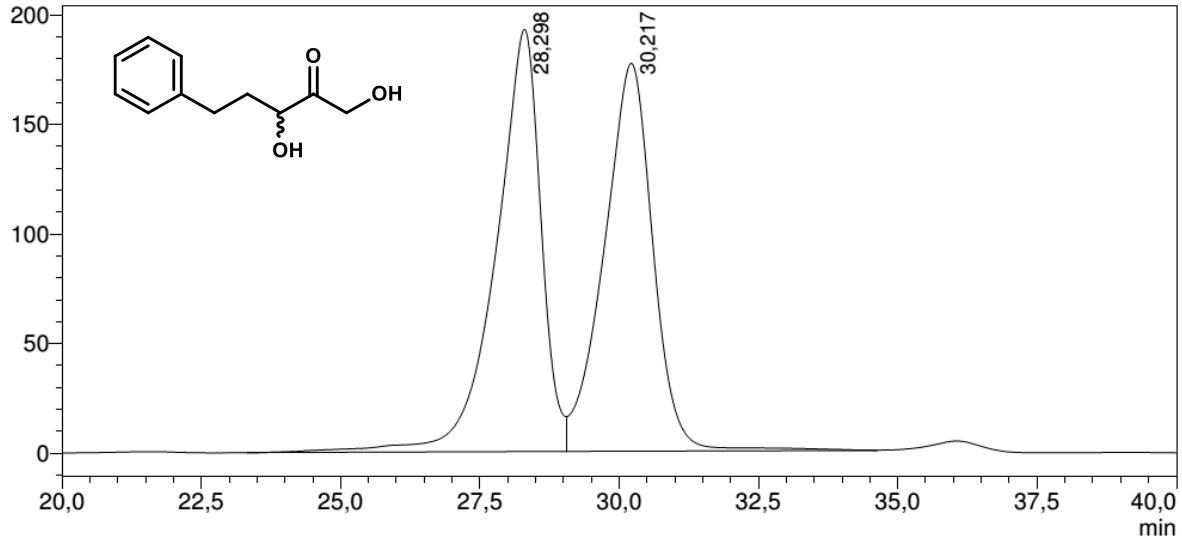
^{1}H NMR spectrum of (*S*)-1,3-dihydroxy-(*N*-Cbz)-5-aminopentan-2-one **(5)** (500 MHz, $CDCl_3$)



^{13}C NMR spectrum of (*S*)-1,3-dihydroxy-(*N*-Cbz)-5-aminopentan-2-one **(5)** (125 MHz, $CDCl_3$)

HPLC Profiles

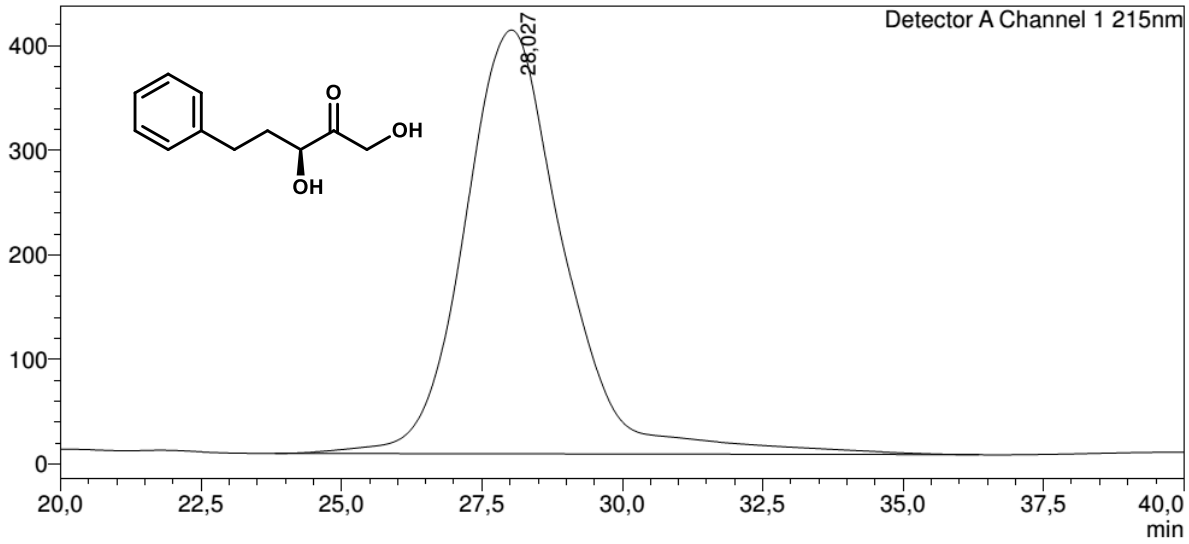
(*RS*)-1, 3-dihydroxy-5-phenylpentan-2-one



Detector A Channel 1 215nm

Peak #	Ret.Time (min)	Area	Height	Conc.
1	28.298	10903091	192733	50.14
2	30.217	10838626	177001	49.85
Total		21741717	369734	

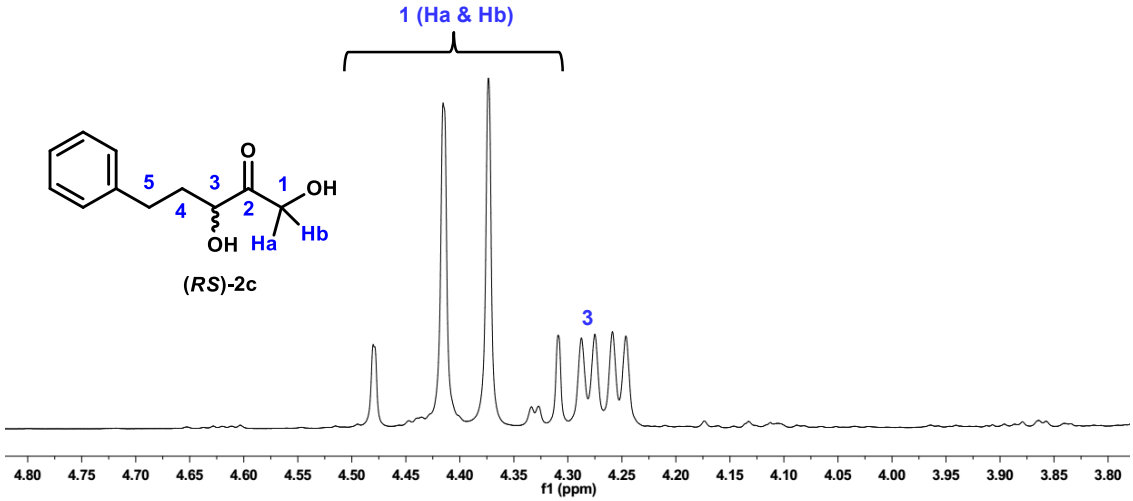
(*S*)-1, 3-dihydroxy-5-phenylpentan-2-one (2c)



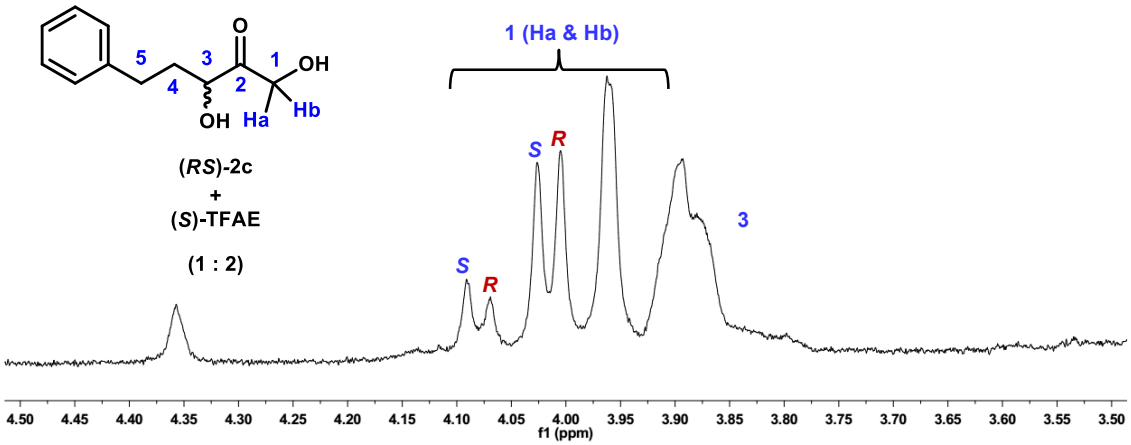
Detector A Channel 1 215nm

Peak #	Ret.Time (min)	Area	Height	Conc.
1	28.027	50190489	405459	100.00
Total		50190489	405459	

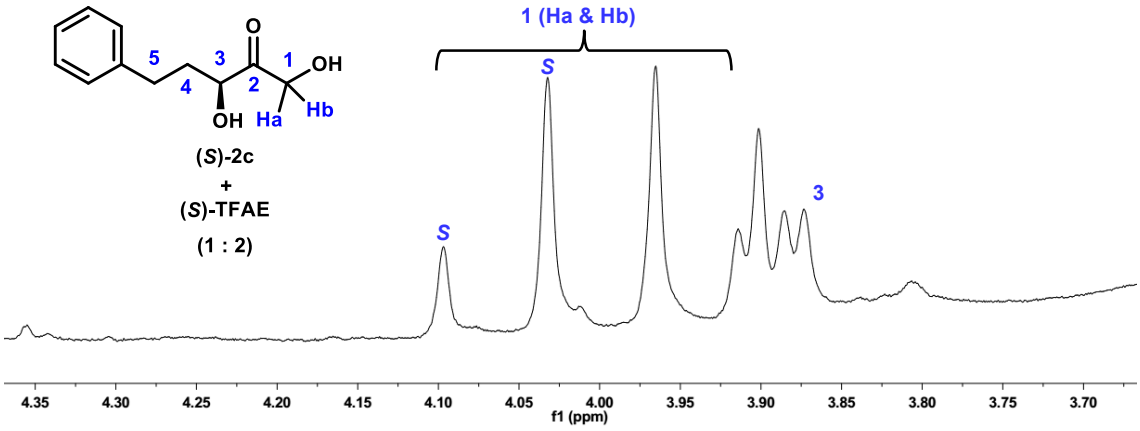
Determination of enantiomeric excess by ^{1}H NMR analysis using chiral solvating agent (*S*)-(+)-2,2,2,-trifluoro-1-(9-anthryl)ethanol [(*S*)-TFAE]



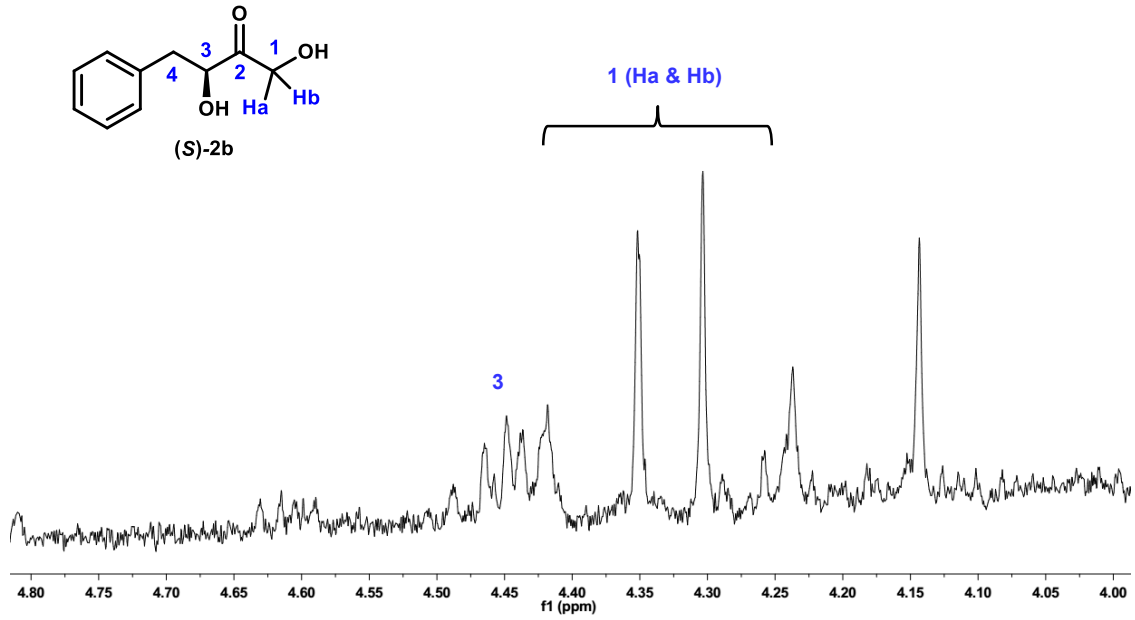
^{1}H NMR spectrum (expansion) of (*RS*)-1,3-dihydroxy-5-phenylpentan-2-one **(2c)** (300 MHz, $CDCl_3$)



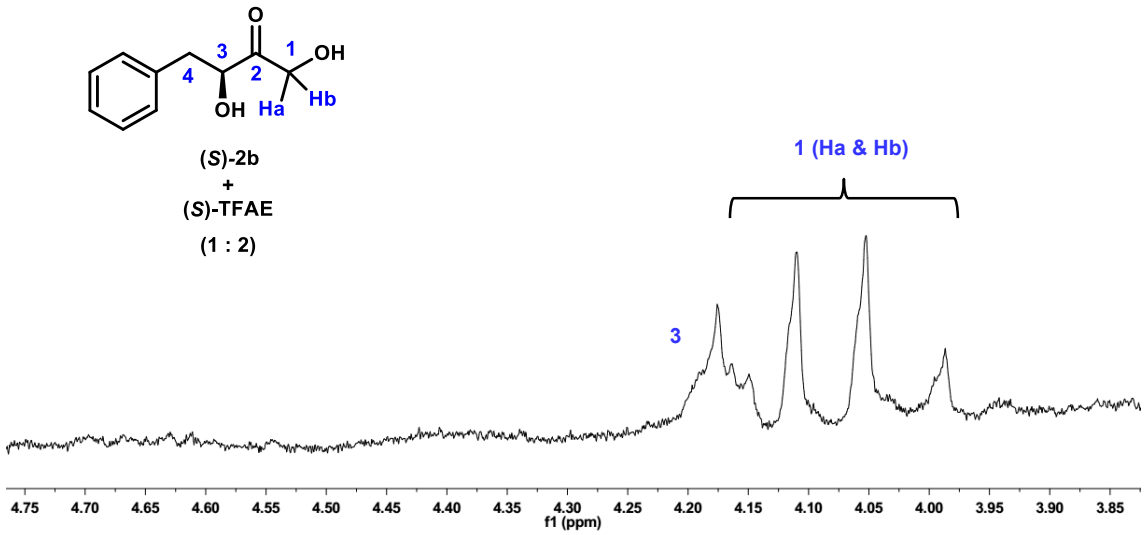
^{1}H NMR spectrum (expansion) of (*RS*)-1,3-dihydroxy-5-phenylpentan-2-one **(2c)** with (*S*)-TFAE (300 MHz, $CDCl_3$)



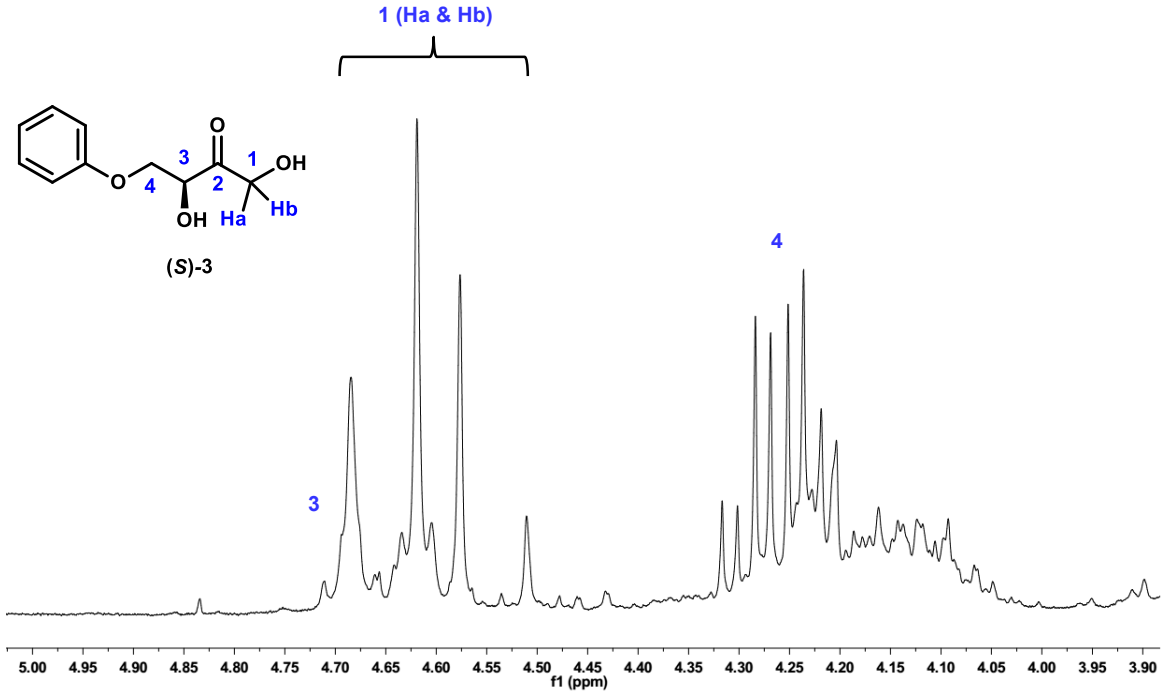
^{1}H NMR spectrum (expansion) of (*S*)-1,3-dihydroxy-5-phenylpentan-2-one **(2c)** with (*S*)-TFAE (300 MHz, $CDCl_3$)



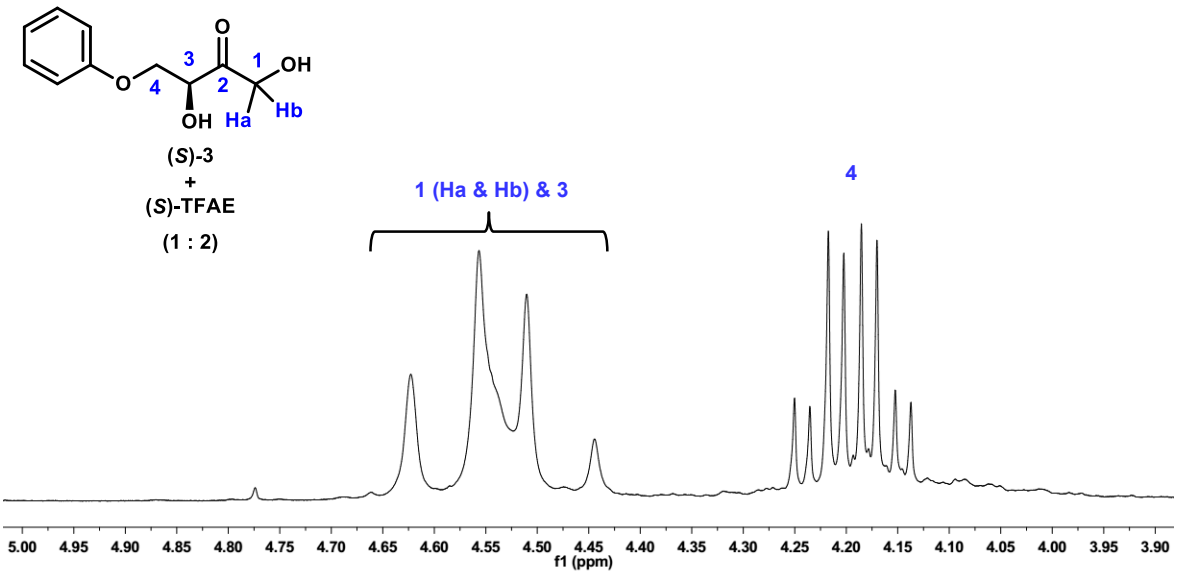
^{1}H NMR spectrum (expansion) of (*S*)-1,3-dihydroxy-4-phenylbutan-2-one **(2b)** (300 MHz, $CDCl_3$)



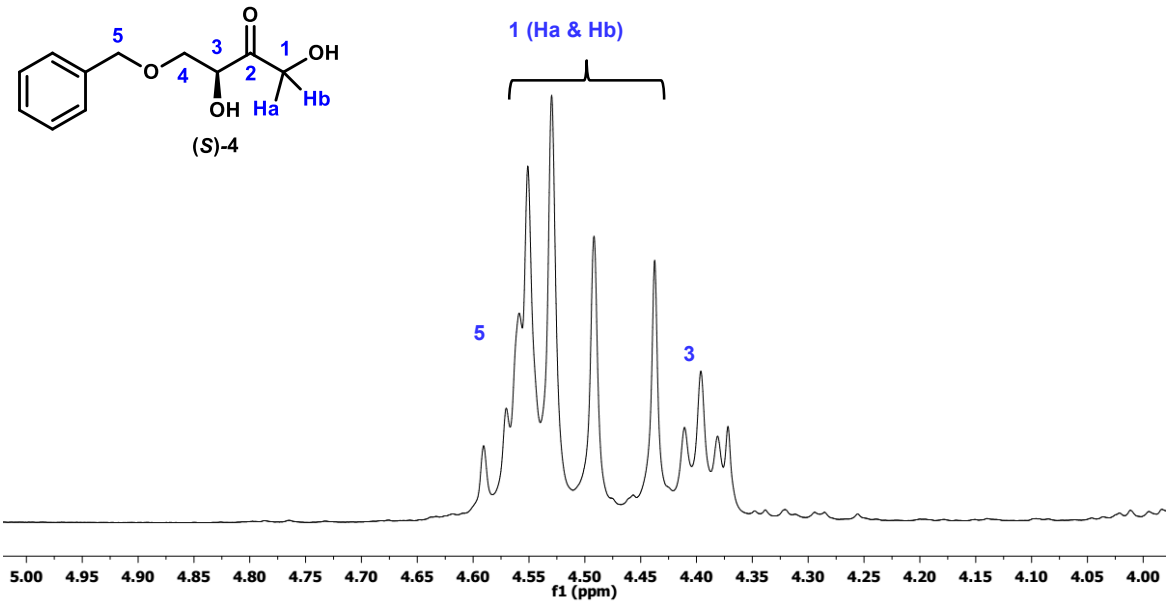
^{1}H NMR spectrum (expansion) of (*S*)-1,3-dihydroxy-4-phenylbutan-2-one **(2b)** with (*S*)-TFAE (300 MHz, $CDCl_3$)



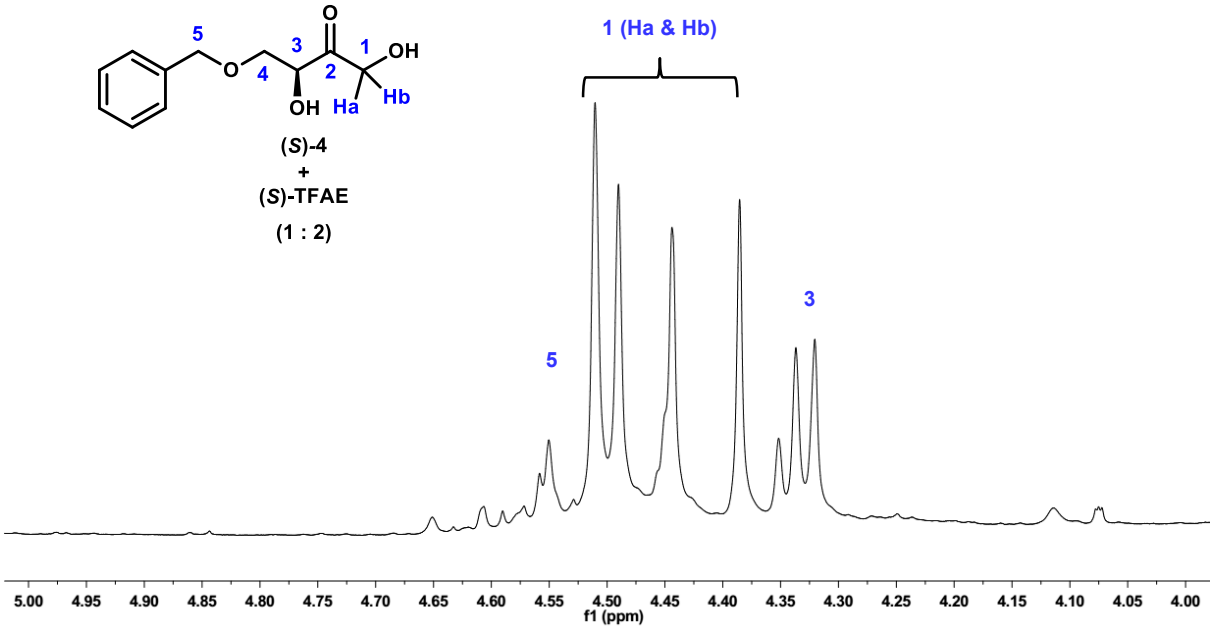
^{1}H NMR spectrum (expansion) of (*S*)-1,3-dihydroxy-4-phenoxybutan-2-one **(3)** (300 MHz, $CDCl_3$)



^{1}H NMR spectrum (expansion) of (*S*)-1,3-dihydroxy-4-phenoxybutan-2-one **(3)** with (*S*)-TFAE (300 MHz, $CDCl_3$)



^{1}H NMR spectrum (expansion) of (*S*)-4-(benzyloxy)-1,3-dihydroxybutan-2-one **(4)** (300 MHz, $CDCl_3$)



^{1}H NMR spectrum (expansion) of (*S*)-4-(benzyloxy)-1,3-dihydroxybutan-2-one **(4)** with (*S*)-TFAE (300 MHz, $CDCl_3$)