Supporting Information

Unlocking Iminium Catalysis in Artificial Enzymes to Create a Friedel-Crafts Alkylase

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1. Supporting Figures

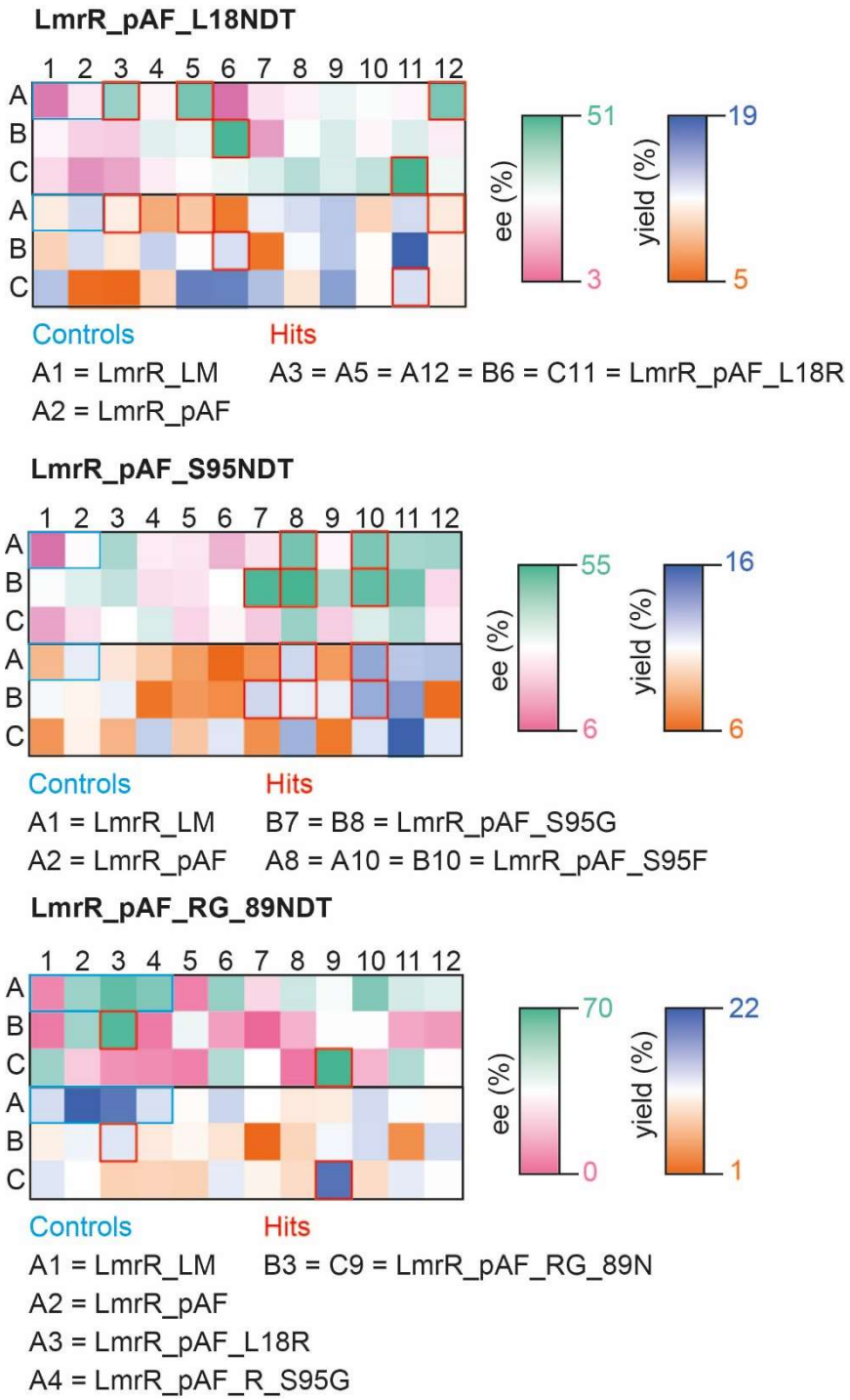

Figure S1: Representative results from library screening

Figures S2-S4: Plots of Michaelis-Menten Kinetics

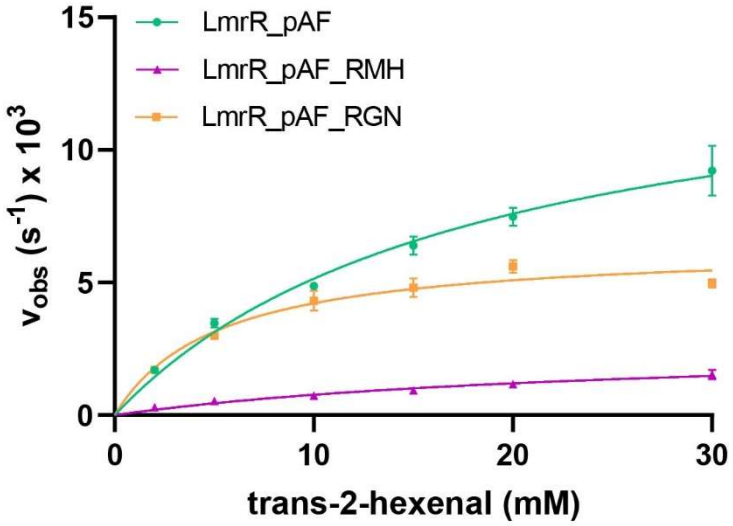

Figure S2: Apparent Michaelis-Menten kinetics of the Friedel-Crafts alkylation of 2-methylindole with trans-2-hexenal for LmrR_pAF, LmrR_pAF_RMH (evolved mutant for hydrazone formation) and LmrR_pAF_RGN (evolved mutant for Friedel-Crafts alkylation). Conditions: 50 mM NaH_2PO_4, 150 mM NaCl, pH 6.5, 1-5 µM protein (dimer) concentration, 1 mM 2-methylindole, 5% DMF, 25 °C. Values are an average of three experiments including both biological and technical replicates. Some error bars are too small to be shown. The rate for each enzyme was determined at 2 mM, 5 mM, 10 mM, 15 mM, 20 mM and 30 mM trans-2-hexenal. The datapoint at 2 mM for LmrR_pAF_RGN (orange squares) is obscured since it overlaps with that of LmrR_pAF (green circles) at the same concentration.

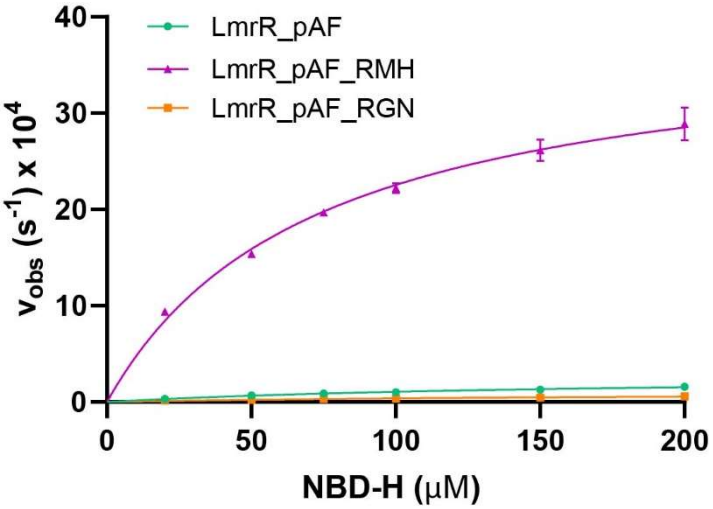

Figure S3: Apparent Michaelis-Menten kinetics of the hydrazone formation of 4-hydroxybenzaldehyde with NDB-H for LmrR_pAF, LmrR_pAF_RMH (evolved mutant for hydrazone formation) and LmrR_pAF_RGN (evolved mutant for Friedel-Crafts alkylation). Conditions: 50 mM NaH_2PO_4, 150 mM NaCl, pH 7.4, 2 µM protein (dimer) concentration, 1-5 µM protein (dimer) concentration, 5 mM 4-hydroxybenzaldehyde, 5% DMF, 25 °C. Values are an average of three experiments including both biological and technical replicates. Some error bars are too small to be shown. This graph includes previously obtained data.[1]

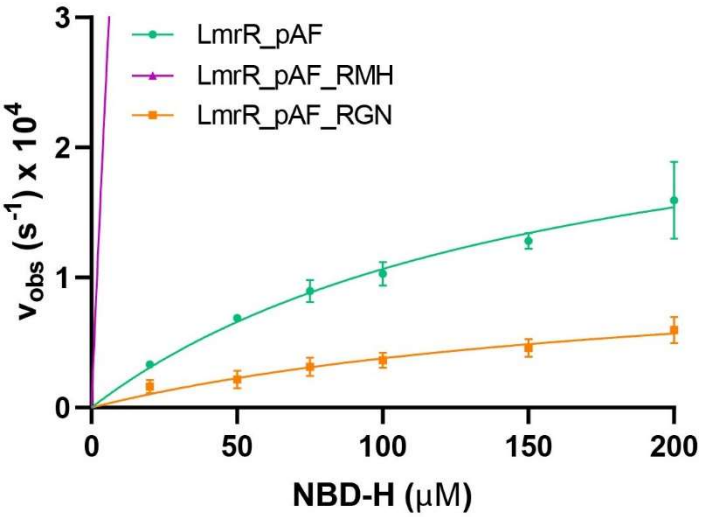

Figure S4: Cropped view of Supplementary Figure S3 to more clearly show the data for LmrR_pAF and LmrR_pAF_RGN. Some error bars are too small to be shown. This graph includes previously obtained data.[1]

2. Supporting Tables

Table S1: Friedel-Crafts catalysis with purified LmrR_pAF (alanine) mutants and control mutants

Mutant	**Yield (%) ± S.D.**	**Δ_{yield} (%)[a]**	***ee* (%) ± S.D.**	**Δ_{ee} (%)[a]**	**$\Delta\Delta\Delta G^{\ddagger}$ (kJ mol^{-1})[b]**
LmrR	2 ± 0	-	N.D.	-	-
LmrR_V15Y	2 ± 0	-	N.D.	-	-
LmrR_V15K	3 ± 0	-	N.D.	-	-
LmrR_V15pAF	42 ± 4	-	45 ± 0	-	-
LmrR_V15pAF_E7A	39 ± 1	-3	45 ± 1	0	+0.04
LmrR_V15pAF_A11L	40 ± 2	-2	49 ± 0	+4	+0.25
LmrR_V15pAF_L18A	**35 ± 5**	**-7**	**29 ± 5**	**-16**	**-0.84**
LmrR_V15pAF_N19A	39 ± 7	-3	49 ± 0	+4	+0.25
LmrR_V15pAF_K22A	**46 ± 3**	**+4**	**36 ± 1**	**-9**	**-0.45**
LmrR_V15pAF_N88A	36 ± 6	-6	49 ± 4	+4	+0.28
LmrR_V15pAF_M89A	37 ± 4	-5	47 ± 2	+2	+0.15
LmrR_V15pAF_A92E	**35 ± 1**	**-9**	**41 ± 0**	**-4**	**-0.21**
LmrR_V15pAF_F93A	**30 ± 4**	**-12**	**47 ± 0**	**+2**	**+0.16**
LmrR_V15pAF_S95A	**38 ± 4**	**-4**	**50 ± 0**	**+5**	**+0.34**
LmrR_V15pAF_S97A	**46 ± 3**	**+4**	**52 ± 1**	**+7**	**+0.42**
LmrR_V15pAF_D100A	22 ± 11	-20	10 ± 0	-35	-1.75

Reaction conditions: 50 mM NaH_2PO_4, 150 mM NaCl, pH 6.5, 1 mM 2-methyl-indole, 5 mM trans-2-hexenal, 8% DMF, 20 μM protein (dimer) concentration, 4 °C and 16 hours reaction time. Values are the average of at least two independent experiments, each conducted in duplicate, errors given are the standard deviation of the results thus obtained. [a] change in *ee* and yields obtained w.r.t. those obtained with LmrR_V15pAF was used to classify the effects of mutations on catalysis. [b] calculated from the enantiomeric ratio according to the equation $\Delta\Delta G^{\ddagger} = -RT\ln(e.r.)$. Entries in bold signify positions chosen as targets for directed evolution.

Table S2: Friedel-Crafts catalysis with LmrR_pAF (alanine) mutants and control mutants in cell-free extract

Mutant	**Δ_{ee} (%)[a]**	**Δ_{yield} (%)[a]**
LmrR	-25 ± 6	-9 ± 3
LmrR_V15Y	-25 ± 6	-10 ± 3
LmrR_V15pAF	0	0
LmrR_V15pAF_E7A	-1 ± 1	-3 ± 1
LmrR_V15pAF_A11L	-4 ± 3	-3 ± 0
LmrR_V15pAF_L18A	**+1 ± 1**	**0 ± 1**
LmrR_V15pAF_N19A	+1 ± 1	-2 ± 0
LmrR_V15pAF_K22A	**-5 ± 3**	**-3 ± 1**
LmrR_V15pAF_N88A	+2 ± 2	-1 ± 2
LmrR_V15pAF_M89A	-16 ± 5	-6 ± 2
LmrR_V15pAF_A92E	**-8 ± 4**	**-3 ± 1**
LmrR_V15pAF_F93A	**-5 ± 0**	**-5 ± 1**
LmrR_V15pAF_S95A	**+1 ± 2**	**+2 ± 1**
LmrR_V15pAF_S97A	**0 ± 3**	**-2 ± 1**
LmrR_V15pAF_D100A	-24 ± 5	-7 ± 2

Reaction conditions: Cell free extract (see methods) 50 mM NaH_2PO_4, 150 mM NaCl, pH 6.5, 1 mM 2-methyl-indole, 15 mM trans-2-hexenal, 8% DMF, 4 ºC and 16 hours reaction time. Values are the average of two independent experiments, each conducted in duplicate, errors given are the standard deviation of the results thus obtained. [a] change in *ee* and yields obtained w.r.t. those obtained with LmrR_V15pAF was used to classify the effects of mutations on catalysis. Absolute yield and enantioselectivity values vary for each independent culture, presumably due to varying expression levels, and thus only the differences with the LmrR_pAF positive control are shown.

Table S3: Summary of library screening results

Round	Library	Hits
1	LmrR_pAF_L18NDT	L18R
1	LmrR_pAF_K22NDT	-
1	LmrR_pAF_A92NDT	A92F, A92Y
1	LmrR_pAF_F93NDT	-
1	LmrR_pAF_S95NDT	S95F, S95G
1	LmrR_pAF_S97NDT	S97F, S97D
2	LmrR_pAF_L18R_K22NDT	-
2	LmrR_pAF_L18R_A92NDT	-
2	LmrR_pAF_L18R_S95G_M8NDT	-
2	LmrR_pAF_L18R_S95G_A11NDT	-
2	LmrR_pAF_L18R_S95G_N19NDT	-
2	LmrR_pAF_L18R_S95G_M89NDT	M89N
2	LmrR_pAF_L18R_S95G_A92NDT	-

Table S4: Friedel-Crafts catalysis with purified LmrR_pAF library hits and recombined mutants and control mutants

Mutant	**Yield (%) ± S.D.**	***ee* (%) ± S.D.**
LmrR_V15pAF	42 ± 4	45 ± 0
LmrR_V15pAF_L18R	58 ± 2	67 ± 0
LmrR_V15pAF_A92F[a]	60	70
LmrR_V15pAF_A92Y[a]	54	57
LmrR_V15pAF_S95F[a]	50	57
LmrR_V15pAF_S95G	46 ± 7	55 ± 1
LmrR_V15pAF_S97F[a]	46	44
LmrR_V15pAF_S97D[a]	36	54
LmrR_V15pAF_L18R_A92F[a]	67	54
LmrR_V15pAF_L18R_S95F[a]	40	17
LmrR_V15pAF_L18R_S95G	70 ± 8	78 ± 0
LmrR_V15pAF_L18R_S95G_M89N	74 ± 2	87 ± 0
LmrR_V15Y_L18R_S95G_M89N	1 ± 0	-21 ± 2
LmrR_V15K_L18R_S95G_M89N	2 ± 0	-11 ± 7

Reaction conditions: 50 mM NaH_2PO_4, 150 mM NaCl, pH 6.5, 1 mM 2-methyl-indole, 5 mM trans-2-hexenal, 8% DMF, 20 μM protein (dimer) concentration, 4 °C and 16 hours reaction time. Values are the average of at least two independent experiments, each conducted in duplicate, except where noted. Errors given are the standard deviation of the results thus obtained. [a] average result from a duplicate experiment

3. Materials and Equipment

Chemicals were purchased from commercial suppliers (Sigma (UK), Acros (Germany), TCI (Belgium/Japan) and Flurochem (UK)) and used without further purification unless specified. Flash column chromatography was performed on silica gel (Silica-P flash silica gel from Silicycle, 0.040-0.063 mm, 230-400 mesh). The unnatural amino acid pAzF was purchased as racemic mixture from Bachem (Switzerland) or as the enantiopure hydrochloride salt from Iris-Biotech (Germany). NMR ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian 400 (400 MHz) spectrometer in $CDCl_3$ or $(CD_3)_2SO$. Chemical shifts values (δ) are denoted in ppm using residual solvent peaks as the internal standard ($CHCl_3$: δ 7.26 for ^{1}H; 77.16 for ^{13}C. $(CD_3)_2SO$: δ 2.50 for ^{1}H; 39.52 for ^{13}C). HPLC analysis was conducted using a Shimadzu LC-10ADVP HPLC equipped with a Shimadzu SPD-M10AVP diode array detector. Plasmids pEVOL-pAzF was obtained from Addgene (pEvol-pAzF was a gift from Prof. Peter Schultz (The Scripps Research Institute)). *E. coli* strains, NEB5-alpha, NEB10-beta and BL21(DE3) (New England Biolabs) were used for cloning and expression. Primers were synthesized by Eurofins MWG Operon (Germany) and Sigma-Aldrich (UK). Plasmid Purification Kits were obtained from QIAGEN (Germany) and DNA sequencing carried out by GATC-Biotech (Germany). Phusion polymerase and DpnI were purchased from New England Biolabs. Gibson cloning was conducted using NEBuilder® HiFi DNA Assembly Master Mix (New England Biolabs). Strep-tactin columns (Strep-Tactin® Superflow® high capacity) and Desthiobiotin were purchased from IBA-Lifesciences (Germany). Concentrations of DNA and protein solutions were determined based on the absorption at 260 nm or 280 nm on a Thermo Scientific Nanodrop 2000 UV-Vis spectrophotometer. UV/Vis absorption spectra and kinetic assays were recorded at 25 ºC on a Jasco V-660 spectrophotometer. UPLC/MS analysis was performed on Waters Acquity Ultra Performance LC with Acquity TQD detector. Water (solvent A) and acetonitrile (solvent B) containing 0.1% formic acid by volume, were used as the mobile phase at a flow rate of 0.3 mL/min. Gradient: 90% A for 2 min, linear gradient to 50% A in 2 min, linear gradient to 20% A in 5 min, followed by 2 min at 5% A. Re-equilibration of the column with 2 min at 90% A.

4. Methods

4.1 Protein Purification

LmrR_pAF variants were produced and purified as previously described[2]. The identity and purity of proteins and the successful reduction of pAzF were determined by mass spectrometry. Protein concentration was determined by correcting the calculated extinction coefficients for LmrR variants for the absorbance of pAF (ε_{280} = 1333 M^{-1} cm^{-1})

4.2 Alanine mutant construction

Where mutations were far separated from position V15 in the LmrR gene, we used previously prepared mutated LmrR plasmids[3] and introduced the V15TAG mutation, in other cases we incorporated the desired mutations into the LrmR_V15TAG gene. Primers are described in section 5, and Phusion polymerase was used. The following PCR protocol was used: (1) initial denaturation at 95 °C for 1 min, (2) 16 cycles of denaturation at 98 °C for 30 s, annealing at 56-68 °C for 30 s, and extension at 72 °C for 5 min, (3) a final extension at 72 °C for 10 min. The resulting PCR product was digested with DpnI for 2 hours at 37 °C and subsequently purified (PCR purification kit, Qiagen). The obtained PCR product (100-200 ng) was transformed into chemically-competent *E. coli* NEB5α cells and spread onto LB agar plates containing ampicillin (100 μg/mL). The plates were incubated at 37 °C overnight and individual colonies were grown in 5 mL LB containing ampicillin (100 μg/mL) overnight. The plasmid DNA was isolated (miniprep kit, Qiagen) and sent for sequencing (GATC Biotech) to confirm correct mutation. The isolated plasmid was then co-transformed with pEVOL_pAzF into chemically-competent *E. coli* BL21(DE3) cells which were spread onto LB agar plates containing ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL). Single colonies from these plates were used for protein expression.

4.3 Library Construction

The previously described pET17b_LmrR_V15X plasmid was used as template for the library construction[1,2]. In addition to an in-frame TAG stop codon at position V15, this variant also features mutations of two lysines (K55D and K59Q), which abrogate the natural DNA-binding ability of LmrR. Randomization at residues lining the active site was achieved by site-directed mutagenesis (QuikChange, Agilent Technologies) starting from pET17b_LmrR_V15X and appropriate primer pairs and Phusion polymerase (see Section 5 of the Supporting Information). The following PCR protocol was used: (1) initial denaturation at 95 °C for 1 min, (2) 16 cycles of denaturation at 98 °C for 30 s, annealing at 56-68 °C for 30 s, and extension at 72 °C for 5 min, (3) a final extension at 72 °C for 10 min. The resulting PCR product was digested with DpnI for 2 hours at 37 °C and subsequently purified (PCR purification kit, Qiagen). The obtained PCR product (100-200 ng) was transformed into chemically-competent *E. coli* NEB10-beta cells (prepared by the Inoue method[4]) and spread onto LB agar plates containing ampicillin (100 μg/mL). The colonies obtained (>100) were scraped off with a Drigalski spatula into 5 mL fresh LB media containing ampicillin (100 μg/mL). The cultures thus obtained were incubated for 2 - 4 hours at 37 °C with 135 rpm shaking. The plasmid DNA was isolated (miniprep kit, Qiagen) and sent for sequencing (GATC Biotech) to confirm library quality. Once library quality was confirmed Q_{pool} ~ 0.6 or greater[5] the plasmid DNA was transformed into chemically-competent *E. coli* BL21 (DE3) cells containing the plasmid pEVOL-pAzF and spread onto LB agar plates containing ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL). After incubation overnight at 37 °C, single colonies were picked and transferred into 96-well plates containing LB media with the same antibiotics. Before the second round the LmrR_V15X_L18R_S95G insert was cloned into the same vector using the Gibson method as described below.

4.4 Gibson Cloning

Before the construction of second-round libraries the LmrR_V15X_L18R_S95G insert was re-cloned into it a fresh vector to prevent the accumulation of detrimental mutations in areas of the plasmid that are not elucidated by sequencing with the T7 primer, after several sequential rounds of QuikChange. We amplified the LmrR_V15X_L18R_S95G insert and the vector from pET17b_LmrR6 using primers described in section 5, Phusion polymerase and the following PCR protocol: (1) initial denaturation at 95 °C for 1 min, (2) 30 cycles of denaturation at 98 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 5 min, (3) a final extension at 72 °C for 10 min. The resulting PCR product was digested with DpnI over-night at 37 °C and subsequently purified (PCR purification kit, Qiagen). The NEBuilder HiFi DNA Assembly Reaction was conducted with the PCR products thus obtained according to the manufacturer protocol. After assembly the product was transformed into chemical competent *E. Coli* NEB5α cells and spread on to LB agar plates containing ampicillin (100 μg/mL) and incubated at 37 °C overnight. Individual colonies were picked into 5 mL LB media with the same antibiotic and grown overnight at 37 °C. The plasmid DNA was isolated (miniprep kit, Qiagen) and sent for sequencing (GATC Biotech).

4.5 Preparation of Cell-Free Extracts in Deep-Well Format

Libraries were prepared as described above. (36 – n) colonies (where n = number of controls, 2 in first round, 4 in second round), obtained from the library preparation, were transferred into 1.5 mL deep well plates containing 500 μL LB media and appropriate antibiotics. In addition to library members, 2 or 4 wells were inoculated with glycerol stocks of controls (LmrR_pAF, LmrR, and LmrR_L18R and LmrR_pAF_R_S95G in the second round). The resulting deep well plates were incubated overnight at 37 °C while shaking at 950 rpm (Titramax 1000 & Incubator 1000, Heidolph). The next morning, 50 μL of the densely grown overnight cultures were transformed into fresh 96-deep well plates containing 1150 μL LB media and appropriate antibiotics. Glycerol (500 μL, 50 % with miliQ water) was added to the remaining overnight culture, mixed thoroughly and stored at - 70 °C. Bacteria were cultured at 37 °C for 5 - 6 hours while shaking at 950 rpm. Subsequently, protein production was induced by addition of 50 μL LB media, containing IPTG (1.2 μL of a 1 M stock solution), arabinose (1.2 μL of a 20% arabinose stock solution) and p-azidophenylalanine at a concentration of 15 mM (final concentrations: IPTG = 1 mM, arabinose = 0.02%, pAzF = 0.6 mM). To avoid precipitation of the unnatural amino acid, pAzF was dissolved by addition of 1 equivalent of base (1 M NaOH) prior to addition to the LB media. Plates were then incubated at 30 °C for 16 hours while shaking (950 rpm) and harvested by centrifugation (3,500 rpm at 4 °C for 15 minutes). After removing the supernatant, cells were washed by addition of 500 μL of buffer A (50 mM sodium phosphate, 150 mM NaCl, pH = 6.5), and the supernatant was again discarded after centrifugations (3,500 rpm for 10 minutes). For the preparation of cell-free extracts, bacteria were resuspended in 300 μL buffer A, containing protease inhibitor (Roche cOmplete), lysozyme (1 mg/mL). DNase I (0.1 mg/mL) and $MgSO_4$ (10 mM) to assist in cell lysis and prevent protein degradation. Resuspended cells were incubated for 2 hours at 30 °C at 800 rpm and then stored until further use at -20 °C. The lysates were defrosted and 30 μL of a TCEP stock solution (100 mM in buffer A, adjusted to pH 6.5 by addition of 6 M NaOH) was added to individual wells. The reduction was initially performed for 2 hours at 30 °C, after which incubation was continued overnight at 4 °C. Subsequently, cell debris was removed by centrifugation (4,000 rpm, 1 hour, 4 °C) and 276 μL of cell-free lysate was transferred into 2 mL microcentrifuge tubes for catalysis, stored at 4 °C and used within 8 hours.

4.6 Friedel-Crafts Catalysis with Cell-Free Extracts and Purified Proteins

Reactions were conducted in 300 μL total volume in a 2 mL microcentrifuge tube. Stock solutions of protein in pH 6.5 PBS buffer (50 mM NaCl, 150 mM NaH_2PO_4) to give the specified final concentration

and the same buffer was added to make up 276 μL volume. For screening of degenerate codon libraries, 276 μL of cell-free lysate was used instead. Stock solutions of indole (25 mM in DMF, 12 μL added, final concentration 1 mM) and enal (125 mM or 675 mM when using cell-free lystate, 12 μL added to give final concentrations of 5 mM or 15 mM with cell free lysate) substrates were added. The microcentrifuge tubes were then mixed by continuous inversion in a cold room as 4 °C for the specified reaction time. After the reaction time had elapsed, $NaBH_4$ solution (60 μL, 20 mg/mL in 0.5 w/v % NaOH) and 3-(3-hydroxyindole) internal standard solution (12 μL, 5 mM in DMF) were added. The micro-centrifuge tubes were mixed by continuous inversion for a further 30 minutes. The reaction products and internal standard were then extracted by vortex mixing with EtOAc (1 mL) and the organic extract was dried over Na_2SO_4, filtered and evaporated to dryness. The residue thus obtained was redissolved by vortex mixing with HPLC grade solvent (heptane:isopropanol 4:1, 90 μL) and analysed by normal phase HPLC to determine yield and enantioselectivity with a 20 μL injection volume.

4.7 Kinetic Characterisation – Hydrazone Formation

Conducted as previously described[1]. One measurement with LmrR_pAF was repeated to obtain results within the error previously determined. Measurements with LmrR_pAF_RGN were conducted in triplicate, one of the triplicate measurements utilised an independently expressed batch of enzyme.

4.8 Kinetic Characterisation – Friedel-Crafts Alkylation

Reactions were conducted as described for catalysis but with 1 mL total volume with a total of 5 vol% DMF, with 1 mM 2-methylindole concentration and varying concentrations of trans-2-hexenal (2 mM, 5 mM, 10 mM, 15 mM, 20 mM and 30 mM) which was distilled by kugelrohr apparatus prior to use. Enzyme concentrations were adjusted so that no reaction aliquot produced a yield above 10 % for any given enzyme mutant and substrate concentration (2 – 20 μM). The microcentrifuge tubes were incubated in an orbital shaker at 25 °C and 300 μL aliquots were removed at three time points (between 15 and 90 minutes depending on the enzyme mutant and enal concentration) and quenched by vortex mixing following addition of $NaBH_4$ solution (60 μL, 20 mg/mL in 0.5 w/v % NaOH) and 3-(3-hydroxyindole) internal standard solution (12 μL, 5 mM in DMF). Products and internal standard were extracted as described for catalysis and the yield was determined in the same manner by normal phase HPLC. Each reaction was conducted in triplicate, one of these triplicate reactions was also conducted using an independently expressed batch of enzyme. Product yields obtained increased linearly across the three time points measured.

5. Primer List

Primer	Sequence
V15TAG_fw	ACCAAT**TAG**ATCCTGCTGAATGTCC
V15TAG_rv	GCAGGAT**CTA**ATTGGTTTGAGCAC
L18A_fw	ATCCTG**GCG**AATGTCCTGAAACAA
L18A_rv	GGACATT**CGC**CAGGATCTAATTGG
N19A_fw	CTGCTG**GCG**GTCCTGAAACAAGGC
N19A_rv	TCAGGAC**CGC**CAGCAGGATCTAATTGGT
K22A_fw	GTCCTG**GCC**CAAGGCGATAACTATGTGT
K22A_rv	GCCTTG**CGG**CAGGACATTCAGCAG
L18NDT_fw	GATCCTG**NDT**AATGTCCTGAAACAAG
L18NDT_rv	GACATT**AHN**CAGGATCTAATTGGTTTGA
K22NDT_fw	TGTCCTG**NDT**CAAGGCGATAACTATGTG
K22NDT_rv	GCCTTG**AHN**CAGGACATTCAGCAG
A92NDT_fw	CGCCTG**NDT**TTCGAATCCTGGAGTCGTG
A92NDT_rv	ATTCGAA**AHN**CAGGCGCATGTTTTCATGGC
F93NDT_fw	GAAAACATGCGTCTGGCC**NDT**GAATCCT
F93NDT_rv	ACACGACTCCAGGATTC**AHN**GGCCAG
S95NDT_fw	CGCCTGGCGTTCGAA**NDT**TGGAGT
S95NDT_rv	TGTCCACACGACTCCA**AHN**TTCGAACG
S97NDT_fw	GCGTTCGAATCCTGG**NDT**CGTGTG
S97NDT_rv	CAATGATTTTGTCCACACG**AHN**CCAGGAT
L18R_K22NDT_fw	TGTCCTG**NDT**CAAGGCGATAACTATGTG
L18R_K22NDT_rv	GCCTTG**AHN**CAGGACATTACGCAG
A11NDT_fw	CTGCGT**NDT**CAAACCAATTAGATCCTG
A11NDT_rv	TGGTTTG**AHN**ACGCAGCATTTCTT
L18R_fw	GATCCTG**CGC**AATGTCCTGAAACAAG
L18R_rv	GGACATT**GCG**CAGGATCTAATTGGTTTG
Gibson_vector_fw	CACCCGCAGTTCGAAAAATAAAAGCTT
Gibson_vector_rv	GGGATTTCGGCACCCATATGTATATCTC
Gibson_insert_fw	GAGATATACATATGGGTGCCGAAATCCC
Gibson_insert_rv	AAGCTTTTATTTTTCGAACTGCGGGTG
L18R_N19NDT_fw	CTGCGT**NDT**GTCCTGAAACAAGGC
L18R_N19NDT_rv	TCAGGAC**AHN**ACGCAGGATCTAATTGG
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S95G_A92NDT_fw	CGCCTG**NDT**TTCGAAGGTTGGAGTCGTG
S95G_A92NDT_rv	CTTCGAA**AHN**CAGGCGCATGTTTTCATGGC
L18R_V15K_fw	ACCAAT**AAA**ATCCTGCGCAATGTCC
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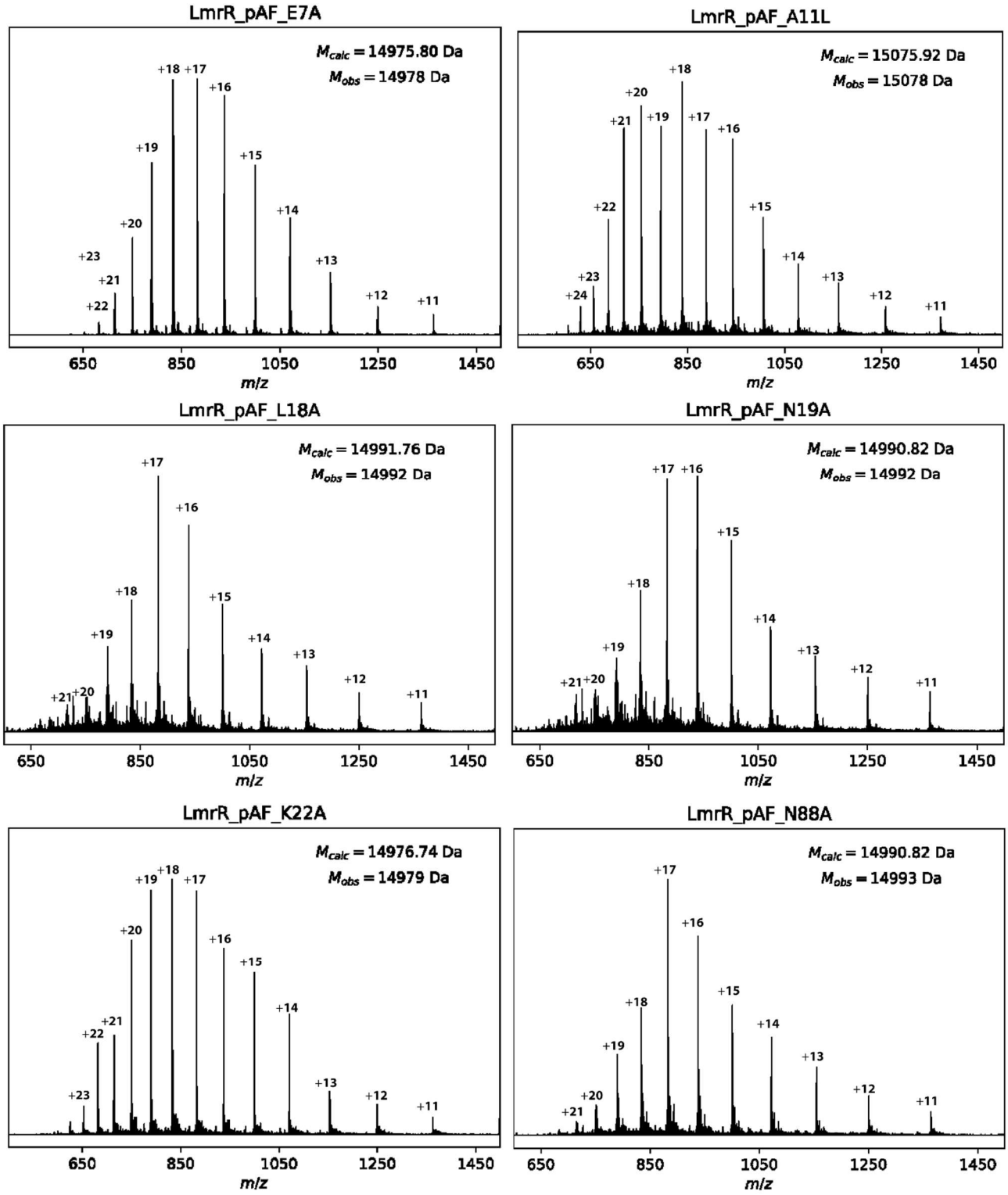
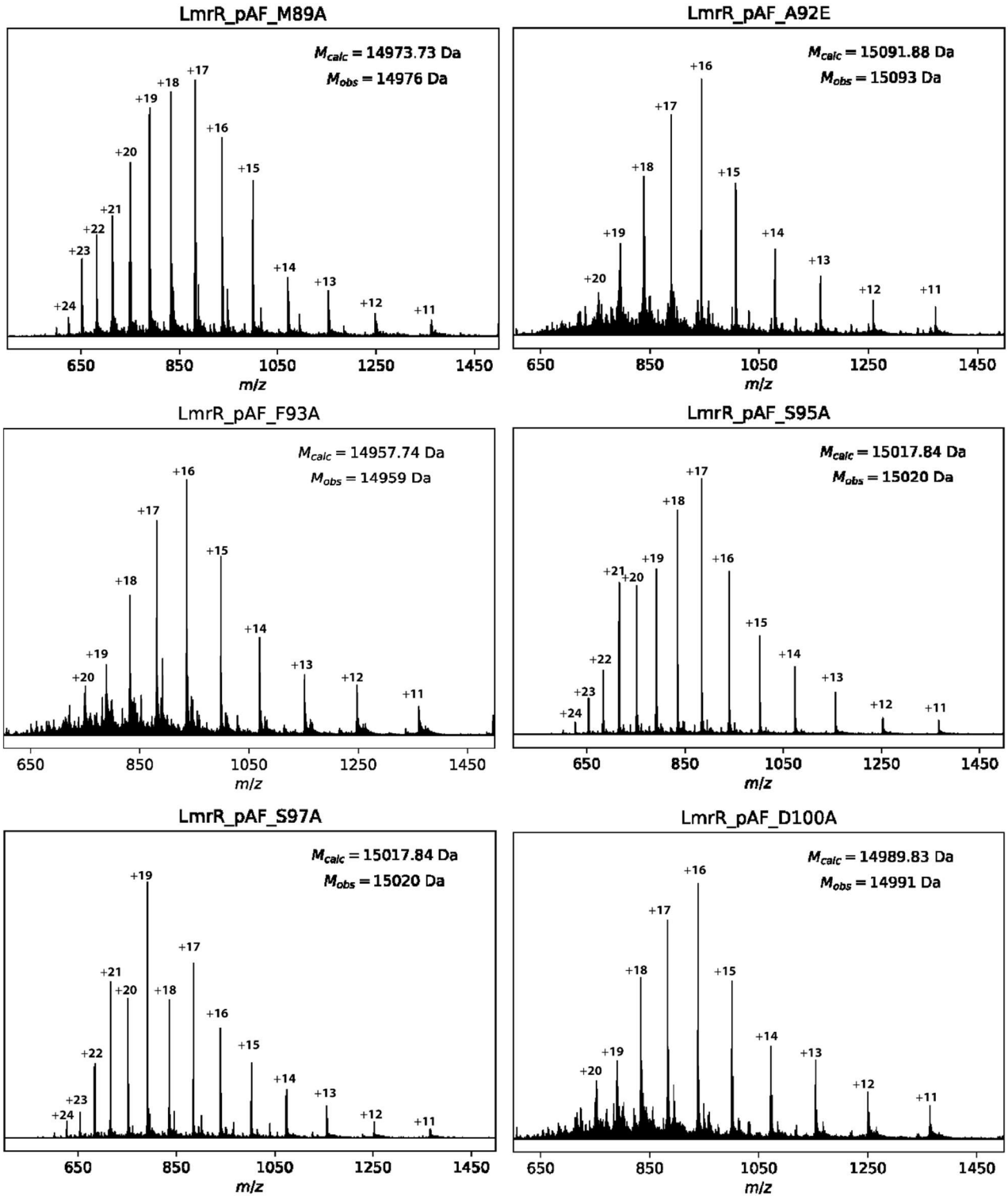
6. Protein Mass Spectrometry

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M_{obs} = 14976 Da
LmrR_pAF_A92E
M_{calc} = 15091.88 Da
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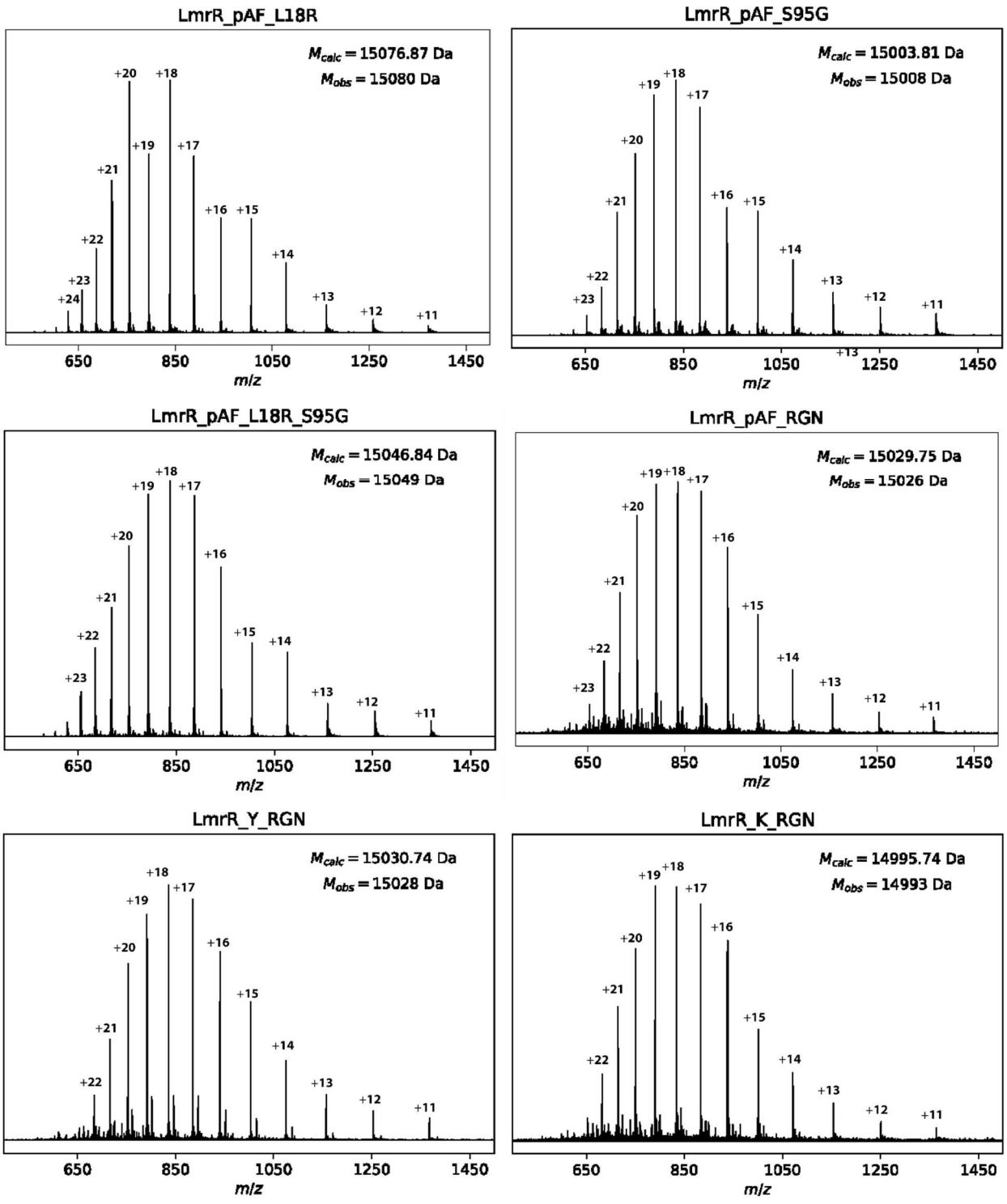
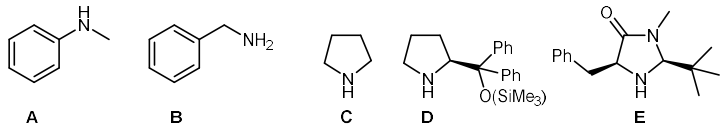
LmrR_pAF_F93A
M_{calc} = 14957.74 Da
M_{obs} = 14959 Da
LmrR_pAF_S95A
M_{calc} = 15017.84 Da
M_{obs} = 15020 Da
LmrR_pAF_S97A
M_{calc} = 15017.84 Da
M_{obs} = 15020 Da
LmrR_pAF_D100A
M_{calc} = 14989.83 Da
M_{obs} = 14991 Da
650
850
1050
1250
1450
m/z


LmrR_pAF_L18R
M_{calc} = 15076.87 Da
M_{obs} = 15080 Da
LmrR_pAF_S95G
M_{calc} = 15003.81 Da
M_{obs} = 15008 Da
LmrR_pAF_L18R_S95G
M_{calc} = 15046.84 Da
M_{obs} = 15049 Da
LmrR_pAF_RGN
M_{calc} = 15029.75 Da
M_{obs} = 15026 Da
LmrR_Y_RGN
M_{calc} = 15030.74 Da
M_{obs} = 15028 Da
LmrR_K_RGN
M_{calc} = 14995.74 Da
M_{obs} = 14993 Da
650
850
1050
1250
1450
m/z

7. Preparation and Characterisation of Reference Products

7.1 Comment on Preparation of reference products



In our hands, racemic reference products could not be efficiently prepared using achiral primary or secondary amines **A**, **B** or **C**. The resulting reaction mixtures obtained using the procedure below with 20 mol % of these catalysts resulted in a complex mixture and an abundance of residual indole as evidenced by TLC. Similar difficulties have been reported elsewhere[6]. Instead, we prepared reference compounds using chiral secondary amines **D** or **E** and diminished the enantioselectivity of the reaction by heating to 50 °C to obtain a mixture of both enantiomers. We demonstrated the separation of the product enantiomers thus obtained on two different chiral stationary phases with normal phase HPLC, measuring very comparable ee in each case, and used these products to prepare calibration curves. We also prepared the racemic products in a crude form without purification using benzylamine **B** and analysed them with the HPLC method used to analyse enzyme catalysed reactions.

7.2 General Procedure for Preparation of Reference Products

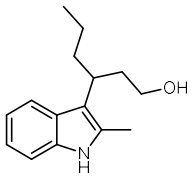
Indole derivatives (0.5 mmol) and enal derivatives (1.5 mmol) and secondary amine **D** (16 mg, 0.05 mmol, general procedure **I**) or secondary amine **E** (12 mg, 0.05 mmol, general procedure **II**) were dissolved in MeOH (2 mL) in a 4 mL dram vial. The reaction mixtures were stirred at 50 °C (general procedure **II**) or ambient temperature (general procedure **I**) and monitored by TLC. After indole consumption was achieved, the reaction was cooled in an ice-bath and $NaBH_4$ (114 mg, 3 mmol) was added portion-wise with stirring. After the addition was complete, the reaction was stirred at ambient temperature for a further 30 minutes. The reaction was extracted with EtOAc (2 x 2 mL) and water/brine (2 mL) and the combined organic extracts were washed with brine (2 mL) and dried over Na_2SO_4. The EtOAc was removed *in vacuo* and the resulting extracts were purified by silica-gel chromatography (heptane:ethyl acetate 6:1-3:1, or pentane:ethyl acetate 3:1). The organic solvent was removed from product containing fractions from the column *in vacuo* to obtain the pure reference products.

7.3 General Procedure for Preparation of Crude Racemic Reference Products

Indole derivatives (0.167 mmol) and enal derivatives (0.5 mmol) and benzylamine **B** (4 μL, 0.033 mmol) were dissolved in methanol (0.7 mL) in a 4 mL dram vial. The reaction mixtures were stirred at 50 °C for 16-21 hr (compounds **3a, 3b, 3c, 3e, 3f, 3g, 3h, 3i**) or 2.5 hr (**3d**) after which time the reaction was cooled to 0 °C in an ice bath and $NaBH_4$ (37 mg, 1 mmol) was added portionwise, after which the reaction was stirred at room temperature for a further 30 minutes. The reaction was extracted with EtOAc (2 x 1 mL) and water/brine (1 mL) and the organic extract was washed with brine (1 mL) and dried over Na_2SO_4. The EtOAc was removed *in vacuo* and the resulting crude product was analysed by HPLC.

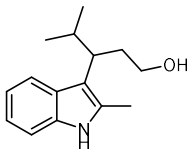
7.4 Characterisation of Reference Products

3-(2-methyl-1H-indol-3-yl)hexan-1-ol (3a)



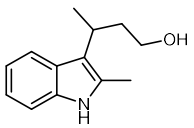
Prepared via general procedure **I** from 2-methyl-indole and trans-2-hexenal, isolated as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.14 – 7.06 (m, 1H), 7.06 – 6.98 (m, 1H), 3.62 – 3.42 (m, 2H), 3.03 – 2.88 (m, 1H), 2.37 (s, 3H), 2.19 – 2.07 (m, 1H), 2.07 – 1.85 (m, 2H), 1.75 – 1.65 (m, 1H), 1.29 – 1.11 (m, 2H), 0.84 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.8, 131.4, 127.5, 120.8, 119.5, 118.9, 113.8, 110.5, 62.1, 38.2, 38.0, 33.6, 21.3, 14.3, 12.3. HRMS (ESI+) calc'd for $C_{15}H_{22}NO$ ($[M+H]^+$) 232.1696; found 232.1695. HPLC Chiracel® OJ-H (heptane:isopropanol 80:20 1 mL/min) 5.7 min (minor); 6.4 min (major) (22 % ee). Chiralcel® AS-H (heptane:isopropanol 90:10 1 mL/min) 5.5 min (minor); 6.1 min (major) (23 % ee).

4-methyl-3-(2-methyl-1H-indol-3-yl)pentan-1-ol (3b)



Prepared via general procedure **II** from 2-methyl-indole and 4-methyl-2-pentenal, isolated as a pale yellow oil. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 10.60 (s, 1H), 7.43 (d, *J* = 7.9 Hz, 1H), 7.20 (d, *J* = 7.9 Hz, 1H), 6.96 – 6.89 (m, 1H), 6.88 – 6.82 (m, 1H), 4.17 (t, *J* = 5.0 Hz, 1H), 3.24 – 3.00 (m, 2H), 2.27 (s, 3H), 2.14 – 1.84 (m, 3H), 1.01 (d, *J* = 6.5 Hz, 3H), 0.65 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.7, 131.7, 127.8, 120.8, 119.7, 119.0, 113.5, 110.4, 62.5, 41.4, 34.9, 32.8, 21.9, 21.8, 12.4. HRMS (ESI+) calc'd for $C_{15}H_{22}NO$ ($[M+H]^+$) 232.1696; found 232.1697. HPLC Chiralcel® AS-H (heptane:isopropanol 90:10 0.5 mL/min) 11.2 min (minor); 12.9 min (major) (74 % ee). Chiracel® OJ-H (heptane:isopropanol 80:20 1 mL/min) 5.7 min (min); 8.4 min (maj) (73 % ee).

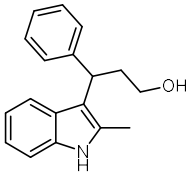
3-(2-methyl-1H-indol-3-yl)butan-1-ol (3c)



Prepared via general procedure **II** from 2-methyl indole and crotonaldehyde, isolated as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.29 – 7.21 (m, 1H), 7.13 – 6.98 (m, 2H), 3.61 – 3.44 (m, 2H), 3.22 – 3.08 (m, 1H), 2.36 (s, 3H), 2.19 – 2.05 (m, 1H), 2.04 – 1.91 (m, 1H), 1.42 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.7, 130.4, 127.4, 120.9, 119.4, 119.0, 115.5, 110.5, 62.1, 39.6, 28.0, 21.5, 12.2. HRMS (ESI+) calc'd for $C_{13}H_{18}NO$ ($[M+H]^+$)

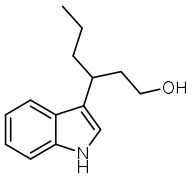
204.1383; found 204.1383. HPLC Chiracel® AS-H (heptane:isopropanol 90:10 0.5 mL/min) 12.8 min (minor); 14.1 min (major) (55 % ee). Chiracel® OJ-H (heptane:isopropanol 80:20 1 mL/min) 8.9 min (minor); 12.5 min (major) (57 % ee).

3-(2-methyl-1H-indol-3-yl)-3-phenylpropan-1-ol (3d)



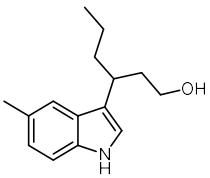
Prepared via general procedure **II** from 2-methyl-indole and cinnamaldehyde, isolated as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.48 (d, J = 7.9 Hz, 1H), 7.37 – 7.32 (m, 2H), 7.25 – 7.19 (m, 3H), 7.17 – 6.93 (m, 3H), 4.41 (t, J = 8.0 Hz, 1H), 3.76 – 3.61 (m, 1H), 3.61 – 3.50 (m, 1H), 2.58 – 2.46 (m, 2H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.1, 135.6, 131.7, 128.4, 127.9, 127.8, 125.9, 121.0, 119.5, 119.4, 113.5, 110.5, 61.8, 38.2, 37.0, 12.4.. HRMS (ESI+) calc'd for $C_{18}H_{20}NO$ ($[M+H]^+$) 266.1539; found 266.1540. HPLC Chiracel® OJ-H (heptane:isopropanol 80:20 1 mL/min) 10.5 min (minor); 12.8 min (major) (31 % ee). Chiracel® OD-H (heptane:isopropanol 85:15 1 mL/min) 25.7 min (major); 29.8 min (minor) (31 % ee).

3-(1H-indol-3-yl)hexan-1-ol (3e)



Prepared via general procedure **II** from indole and trans-2-hexenal, isolated as a colourless oil. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 10.74 (s, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.05 (d, J = 2.3 Hz, 1H), 7.03 (ddd, J = 8.2, 6.9, 1.2 Hz, 1H), 6.92 (ddd, J = 8.0, 6.9, 1.1 Hz, 1H), 3.36 – 3.22 (m, 2H), 3.00 – 2.89 (m, 1H), 1.81 (q, J = 7.0 Hz, 2H), 1.73 – 1.55 (m, 2H), 1.22 – 1.11 (m, 2H), 0.81 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.7, 127.1, 122.0, 121.2, 119.8, 119.7, 119.2, 111.3, 61.9, 39.0, 38.7, 33.6, 20.9, 14.3. HRMS (APCI+) calc'd for $C_{14}H_{20}NO$ ($[M+H]^+$) 218.1539; found 218.1541. HPLC Chiracel® OD-H (heptane:isopropanol 85:15 1 mL/min) 12.0 min (major); 14.2 min (minor) (68 % ee). Chiracel® OJ-H (heptane:isopropanol 80:20 1 mL/min) 9.8 min (minor); 11.5 min (major) (67 % ee).

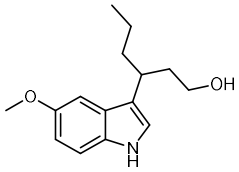
3-(5-methyl-1H-indol-3-yl)hexan-1-ol (3f)



Prepared via general method **II** from 5-methyl-indole and trans-2-hexenal, isolated as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.43 (s, 1H), 7.25 (d, J = 8.3 Hz, 1H), 7.01 (dd, J = 8.3, 1.6 Hz, 1H), 6.93 (d, J = 2.4 Hz, 1H), 3.65 – 3.50 (m, 2H), 3.10 – 2.93 (m, 1H), 2.46 (s, 3H), 2.06 – 1.95 (m, 2H), 1.81 – 1.66 (m, 2H), 1.31 – 1.21 (m, 2H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.1, 128.4, 127.3, 123.7, 121.4, 119.3, 119.2, 111.0, 61.9, 38.9, 38.7, 33.7, 21.7, 21.0, 14.3. HRMS

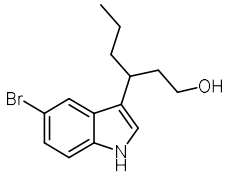
(ESI+) calc'd for $C_{15}H_{22}NO$ ($[M+H]^+$) 232.1696; found 232.1698. HPLC Chiracel® AS-H (heptane:isopropanol 90:10 0.5 mL/min) 12.2 min (minor); 13.1 min (major) (68 % ee). Chiracel® OD-H (heptane:isopropanol 85:15 1 mL/min) 10.5 min (major); 11.1 min (minor) (68 % ee).

3-(5-methoxy-1H-indol-3-yl)hexan-1-ol (3g)



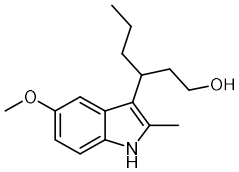
Prepared via general method **II** from 5-methoxy-indole and trans-2-hexenal, isolated as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.23 (d, J = 8.8 Hz, 1H), 7.10 (d, J = 2.4 Hz, 1H), 6.93 (d, J = 2.4 Hz, 1H), 6.86 (dd, J = 8.8, 2.4 Hz, 1H), 3.87 (s, 3H), 3.66 – 3.53 (m, 2H), 3.07 – 2.95 (m, 1H), 2.04 – 1.92 (m, 2H), 1.80 – 1.63 (m, 2H), 1.34 – 1.21 (m, 2H), 0.87 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.7, 131.9, 127.5, 122.1, 119.4, 112.0, 111.9, 101.8, 61.8, 56.1, 38.8, 38.6, 33.5, 20.9, 14.3. HRMS (ESI+) calc'd for $C_{15}H_{22}NO_2$ ($[M+H]^+$) 248.1645; found 248.1648. HPLC Chiracel® OD-H (heptane:isopropanol 85:15 1 mL/min) 13.9 min (major); 15.1 min (minor) (68 % ee). Chiracel® OB-H (heptane:isopropanol 90:10 1 mL/min) 14.4 min (major); 19.0 min (minor) (69 % ee).

3-(5-bromo-1H-indol-3-yl)hexan-1-ol (3h)



Prepared via general method **II** from 5-bromo-indole and trans-2-hexenal, isolated as a colourless oil. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 11.00 (s, 1H), 7.67 (d, J = 1.9 Hz, 1H), 7.29 (d, J = 8.6 Hz, 1H), 7.17 – 7.10 (m, 2H), 3.35 – 3.21 (m, 2H), 2.99 – 2.87 (m, 1H), 1.87 – 1.69 (m, 2H), 1.68 – 1.54 (m, 2H), 1.23 – 1.09 (m, 2H), 0.81 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.3, 128.8, 124.9, 122.4, 122.2, 119.5, 112.8, 112.6, 61.7, 38.8, 38.6, 33.4, 20.9, 14.2. HRMS calc'd for $C_{14}H_{18}BrNO$ ($[M+H]^+$) 296.0645 and 298.0624; found 269.0655 and 298.0631. HPLC Chiracel® AS-H (heptane:isopropanol 90:10 0.5 mL/min) 14.0 min (minor); 15.3 min (major) (67 % ee). Chiracel® OD-H (heptane:isopropanol 85:15 1 mL/min) 10.2 min (major); 11.8 min (minor) (67 % ee).

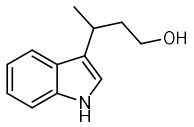
3-(5-methoxy-2-methyl-1H-indol-3-yl)hexan-1-ol (3i)



Prepared via general method **II** from 5-methoxy-2-methyl-indole and trans-2-hexenal, isolated as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.15 (d, J = 8.7 Hz, 1H), 7.05 (d, J = 2.4 Hz, 1H), 6.75 (dd, J = 8.7, 2.4 Hz, 1H), 3.84 (s, 3H), 3.60 – 3.41 (m, 1H), 3.02 – 2.86 (m, 1H), 2.35 (s, 3H), 2.16 – 2.06 (m, 1H), 2.03 – 1.83 (m, 2H), 1.75 – 1.63 (m, 1H), 1.26 – 1.14 (m, 3H), 0.85 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.5, 132.4, 131.0, 128.0, 113.6, 110.9, 109.9, 102.6, 62.1, 56.2, 38.0, 37.8, 33.5, 21.3, 14.3, 12.4. HRMS calc'd for $C_{16}H_{24}NO_2$ ($[M+H]^+$) 262.1801; found 262.1804. HPLC

Chiracel® OD-H (heptane:isopropanol 85:15 1 mL/min) 11.4 min (major); 17.7 min (minor) (64 % ee). Chiracel® AS-H (heptane:isopropanol 90:10 0.5 mL/min) 12.3 min (minor); 14.1 min (major) (62 % ee).

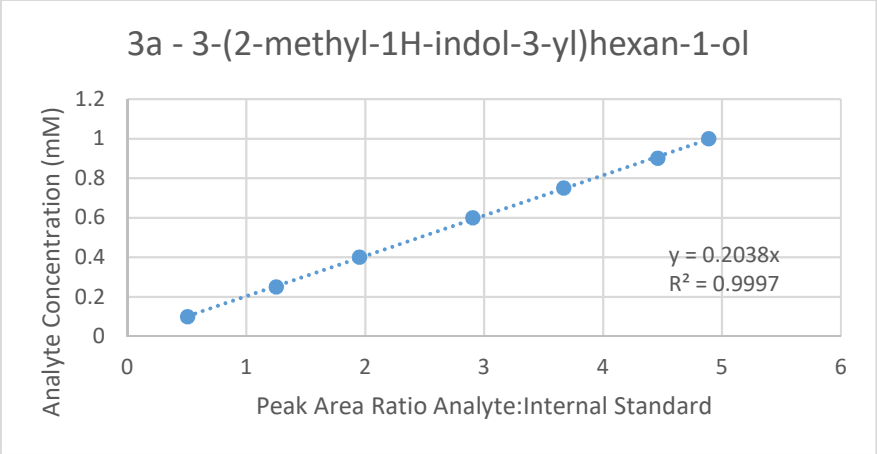
3-(1H-indol-3-yl)-butan-1-ol – 3j

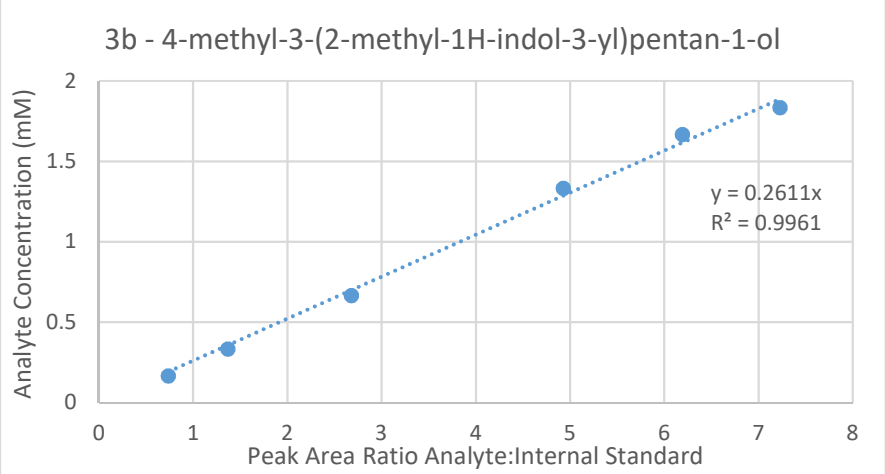


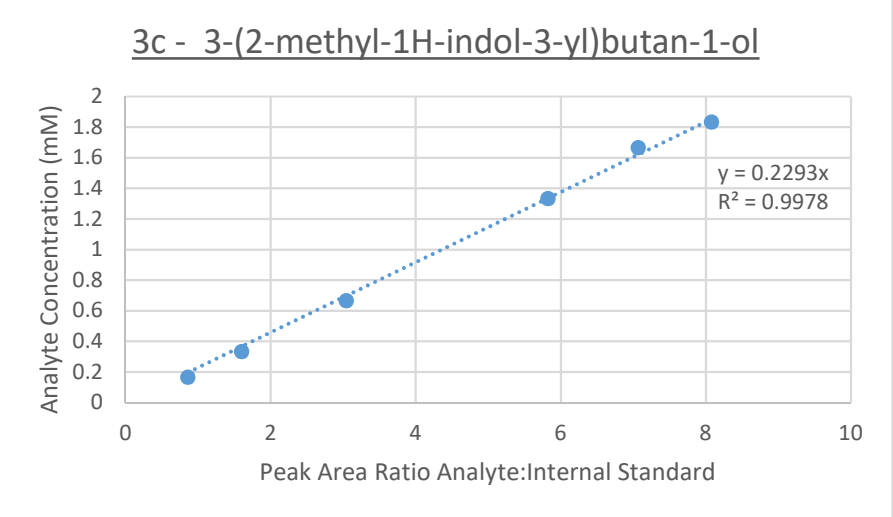
Prepared according to literature procedure with minor moditications[7]. (2S,5S)-5-benzyl-2-tert-butyl-3-methyl-imidazolidin-4-one (62 mg, 0.25 mmol) was dissolved in CH_2Cl_2 (2.25 mL) and isopropanol (0.25 mL) at room temperature and 2,4-dinitrobenzoic acid (53 mg, 0.25 mmol) was added. After 5 minutes stirring crotonaldehyde (100 µL, 1.25 mmol) was added. After a further 10 minutes stirring indole (146 mg, 1.25 mmol) was added. After 3 days of stirring at room temperature, the reaction was diluted with methanol (2.5 mL) and sodium borohydride (142 mg, 3.75 mmol) was added portionwise at 0ºC. After addition was finished the reaction was left to stir for a futher 30 minutes and was then quenched with water (5 mL) and brine (5 mL) and extracted with ethyl acetate (2 x 25 mL). The combined organic extracts where washed with water (10 mL) and dried over sodium sulfate. The ethyl acetate was removed *in vacuo* and the crude material thus obtained was purified by silica-gel chromatography (pentane:EtOAc 5:1 to 4:1). The product was obtained as a colourless oil. The ^{1}H NMR was consistent with the literature[8]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.67 (d, J = 7.9 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.19 (t, J = 7.5 Hz, 1H), 7.11 (t, J = 7.4 Hz, 1H), 7.00 (d, J = 2.3 Hz, 1H), 3.74 – 3.61 (m, 2H), 3.31 – 3.17 (m, 1H), 2.13 – 2.00 (m, 1H), 2.00 – 1.89 (m, 1H), 1.40 (d, J = 7.0 Hz, 3H). The order of elution on chiral HPLC was consistent with the literature indicating that the product was enriched in the R isomer. Chiracel® OD-H (heptane:isopropanol 85:15 1 mL/min) 14.3 min (major, R-isomer); 15.9 min (minor, S isomer) 70% ee (obtained at room temperature) . Literature: Chiracel® OD-H with OD guard column (hexanes:ethanol 90:10 1 mL/min) 17.6 min (major, R-isomer); 20.2 min (minor, S-isomer) 91 % ee (obtained at -60 ºC). The separation method and purity were also validated by separation on another stationary phase: Chiracel® OJ-H (heptane:isopropanol 80:20 1 mL/min) 16.4 min (minor) 18.9 (major) 70 % ee.

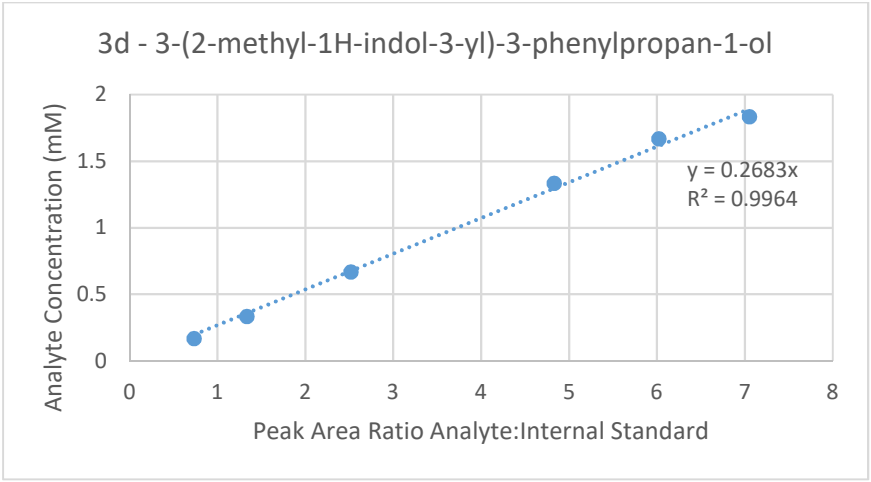
The order of elution obtained with artificial enzymes LmrR_pAF and LmrR_pAF_RGN was opposite to that obtained with (2S,5S)-5-benzyl-2-tert-butyl-3-methyl-imidazolidin-4-one indicating that these enzymes produce the (S)-isomer as the major product.

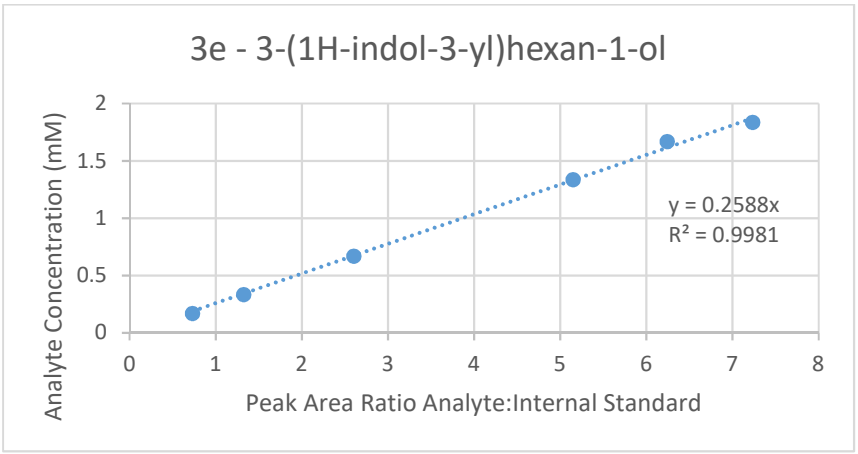
8. Calibration Curves

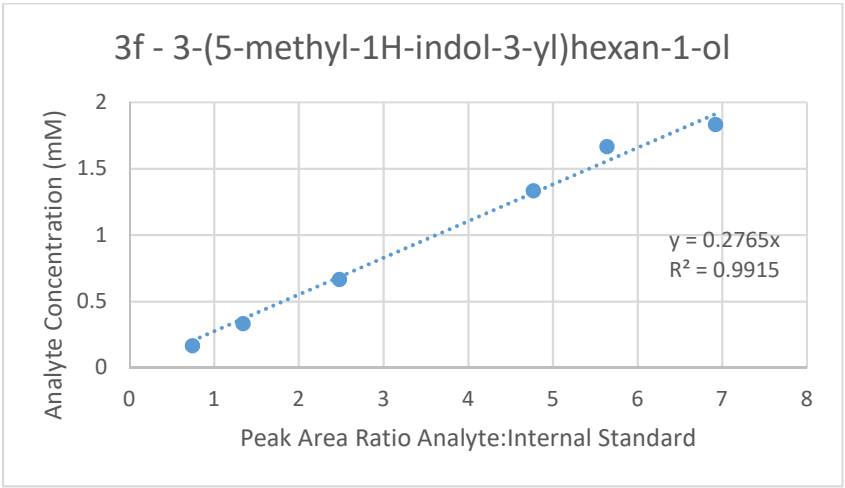


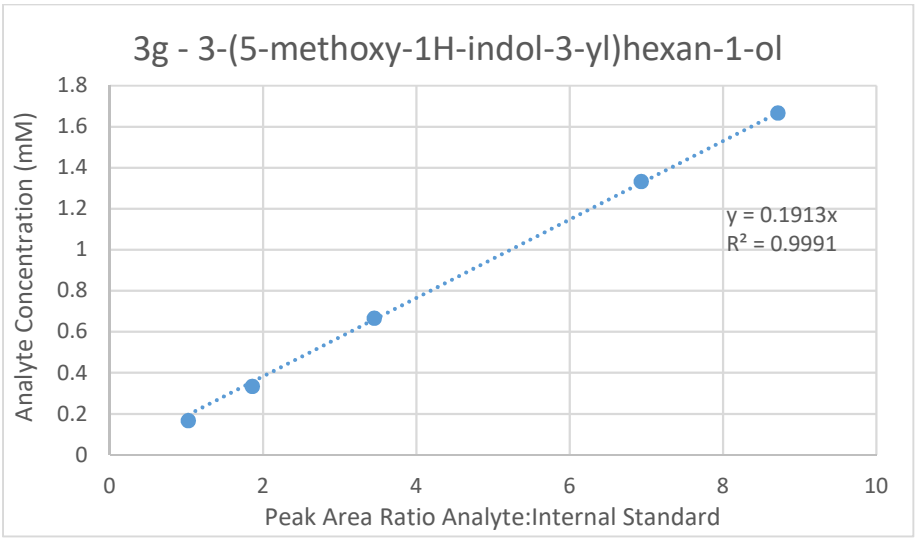


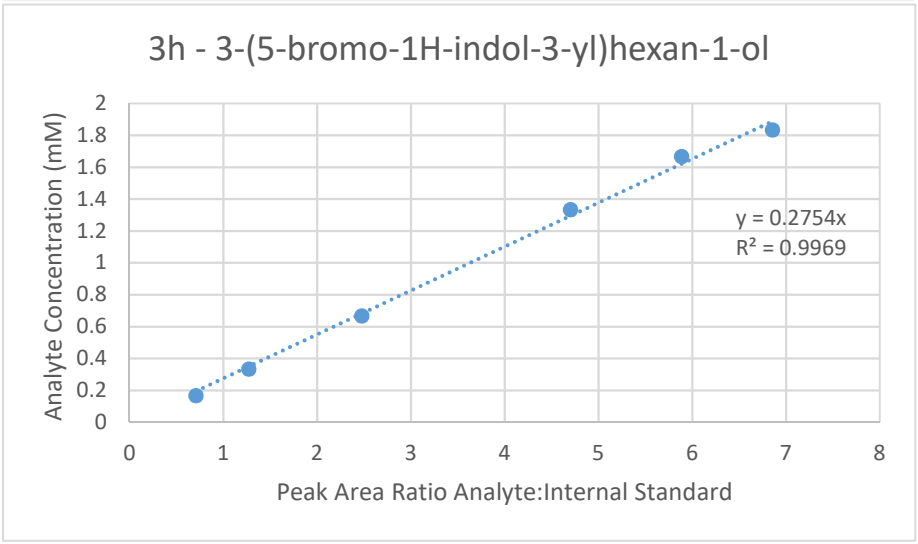


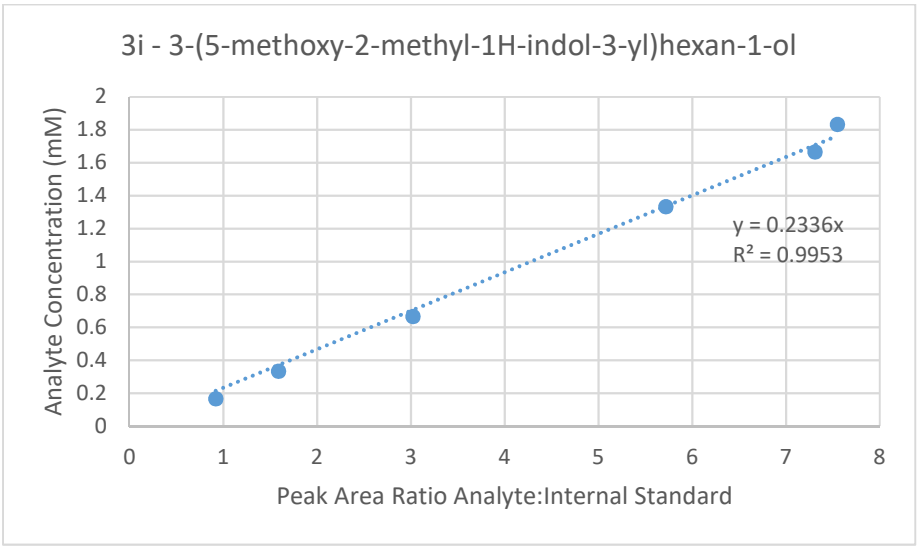

3d - 3-(2-methyl-1H-indol-3-yl)-3-phenylpropan-1-ol
Analyte Concentration (mM)
Peak Area Ratio Analyte:Internal Standard
y = 0.2683x
R² = 0.9964

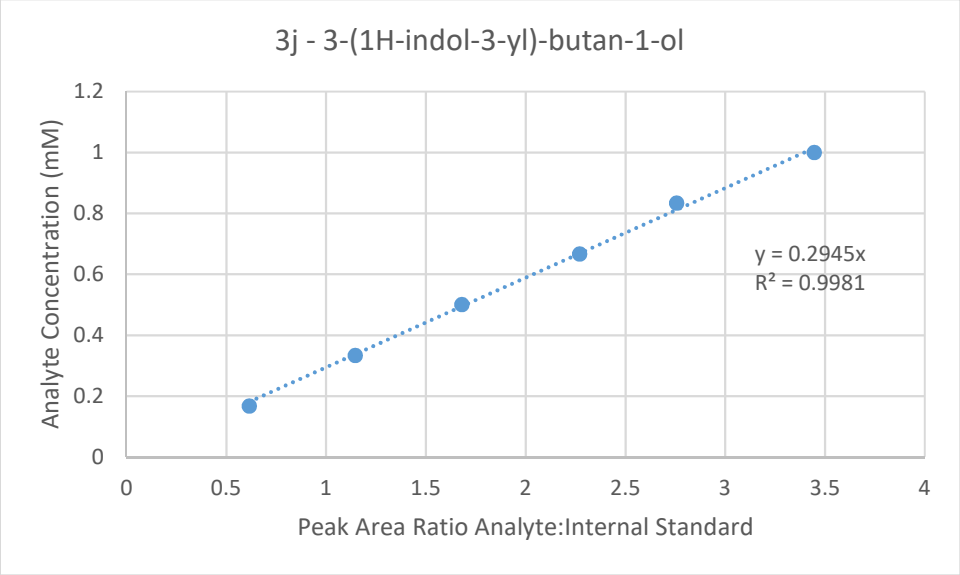

3e - 3-(1H-indol-3-yl)hexan-1-ol
Analyte Concentration (mM)
Peak Area Ratio Analyte:Internal Standard
y = 0.2588x
R² = 0.9981


3f - 3-(5-methyl-1H-indol-3-yl)hexan-1-ol
Analyte Concentration (mM)
Peak Area Ratio Analyte:Internal Standard
y = 0.2765x
R² = 0.9915


3g - 3-(5-methoxy-1H-indol-3-yl)hexan-1-ol
Analyte Concentration (mM)
Peak Area Ratio Analyte:Internal Standard
y = 0.1913x
R² = 0.9991


3h - 3-(5-bromo-1H-indol-3-yl)hexan-1-ol
Analyte Concentration (mM)
Peak Area Ratio Analyte:Internal Standard
y = 0.2754x
R² = 0.9969

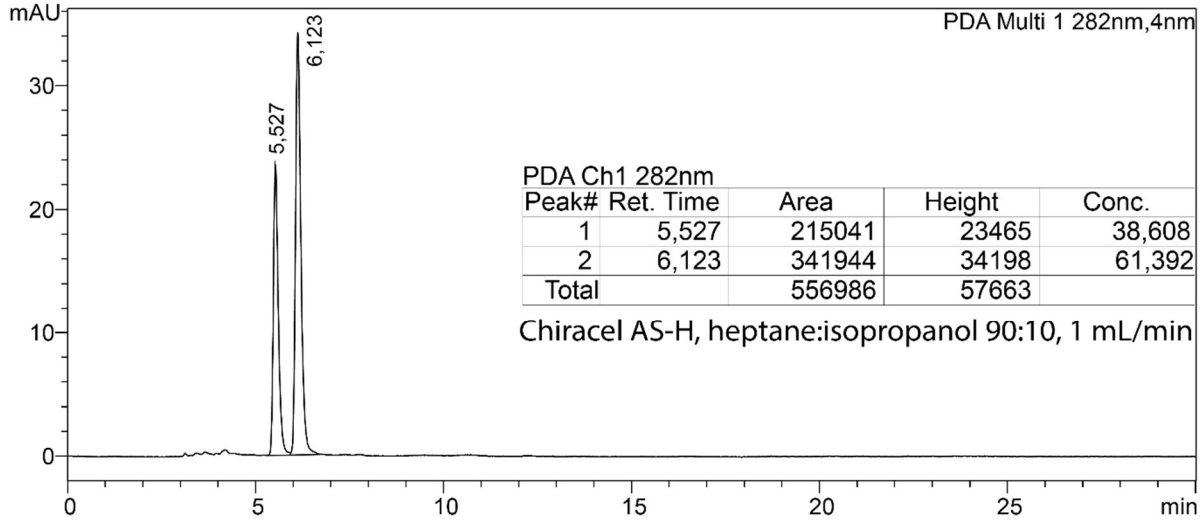

3i - 3-(5-methoxy-2-methyl-1H-indol-3-yl)hexan-1-ol
Analyte Concentration (mM)
Peak Area Ratio Analyte:Internal Standard
y = 0.2336x
R² = 0.9953

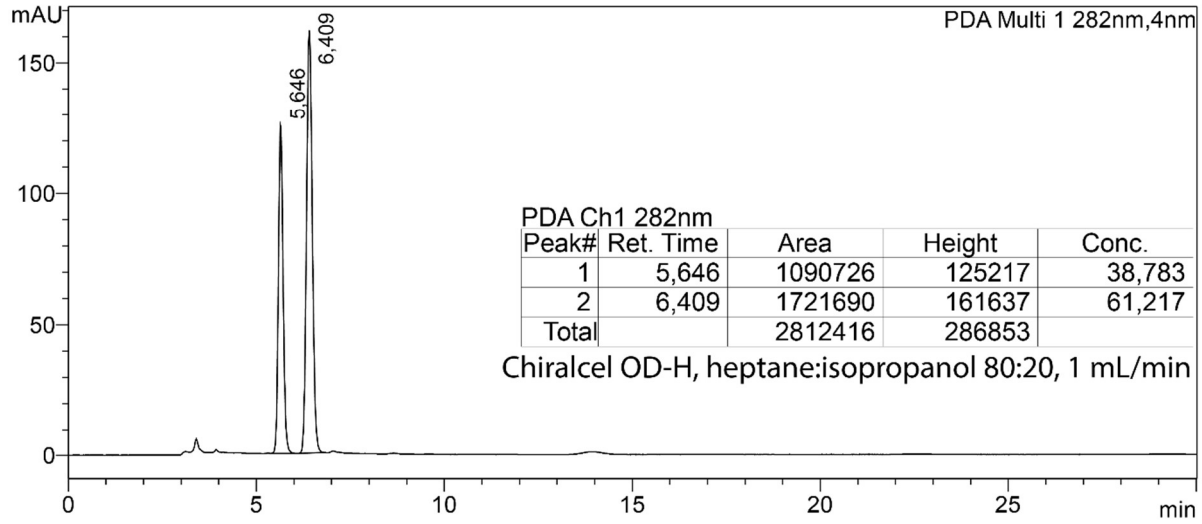

3j - 3-(1H-indol-3-yl)-butan-1-ol
Analyte Concentration (mM)
1.2
1
0.8
0.6
0.4
0.2
0
0
0.5
1
1.5
2
2.5
3
3.5
4
Peak Area Ratio Analyte:Internal Standard
y = 0.2945x
R² = 0.9981

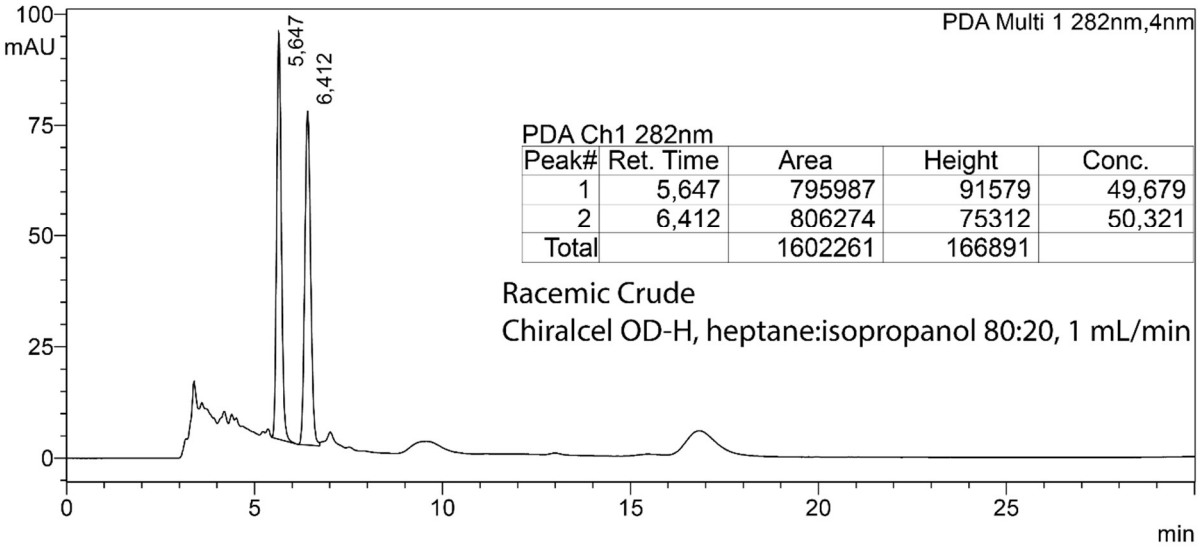
9. HPLC Chromatograms

3-(2-methyl-1H-indol-3-yl)hexan-1-ol (3a)

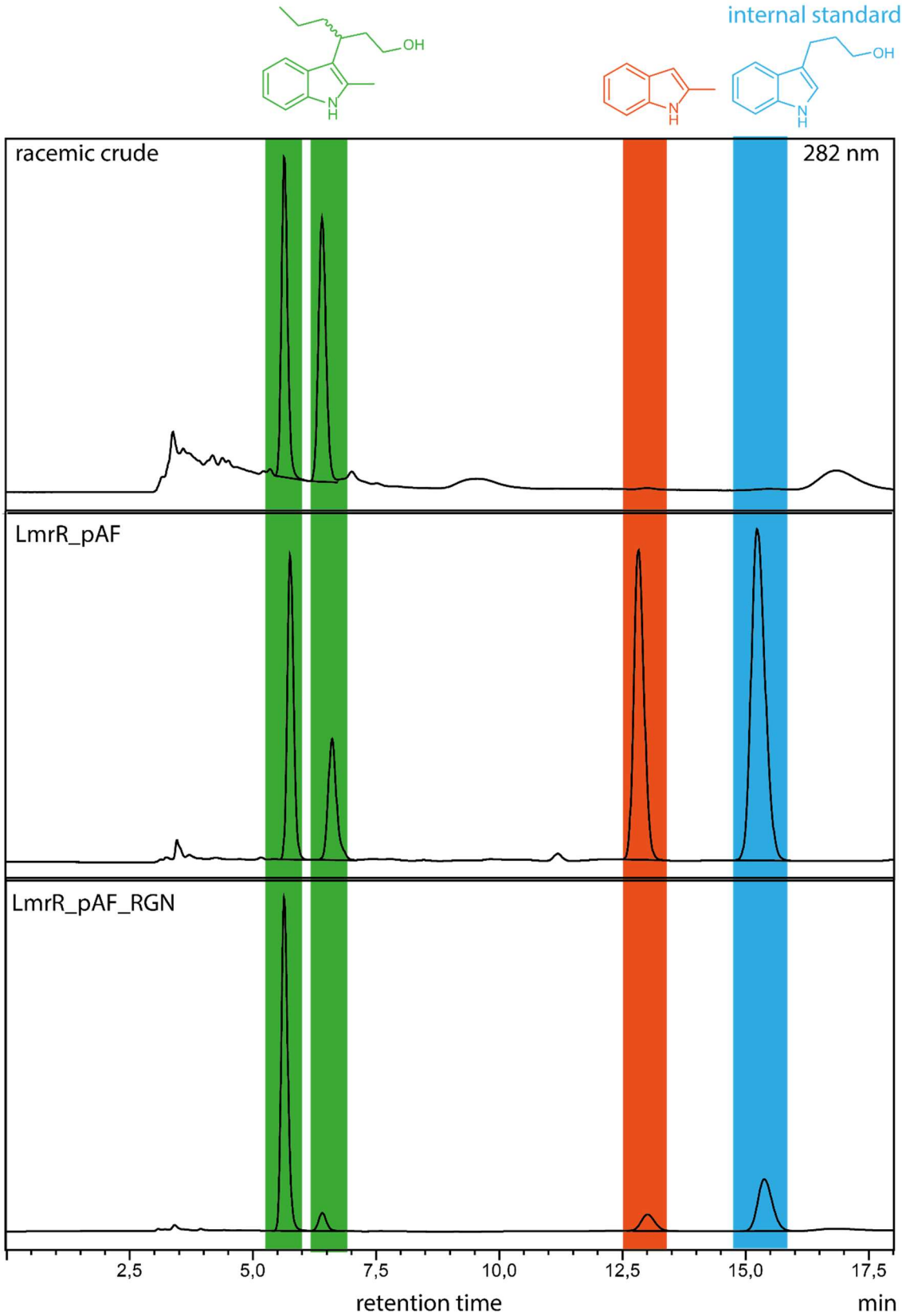
Reference compound and racemic crude product:







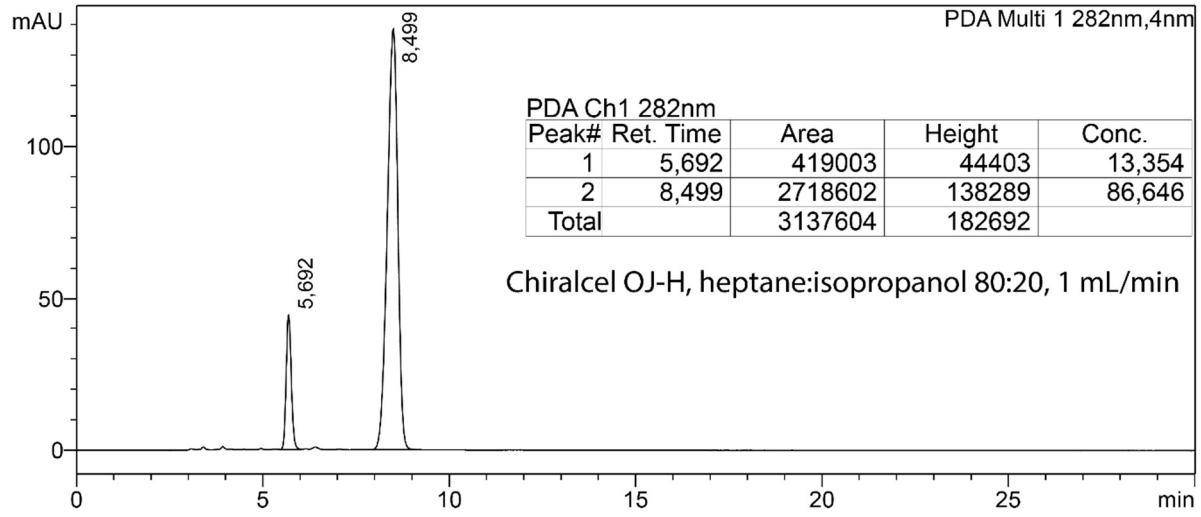
Artificial Enzyme catalysed reactions: Chiracel OJ-H, heptane isopropanol 80:20, 1 mL/min

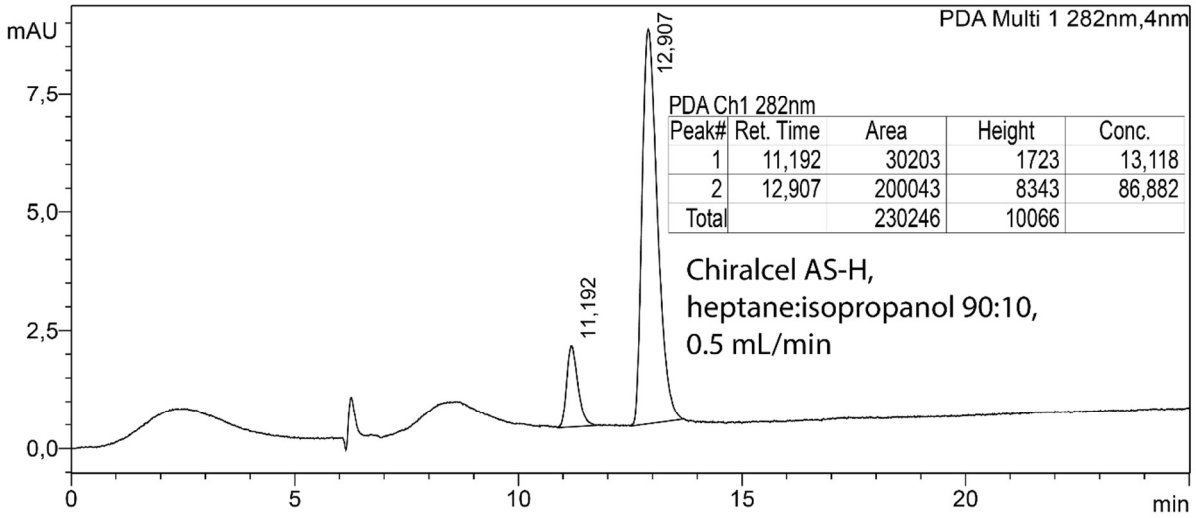


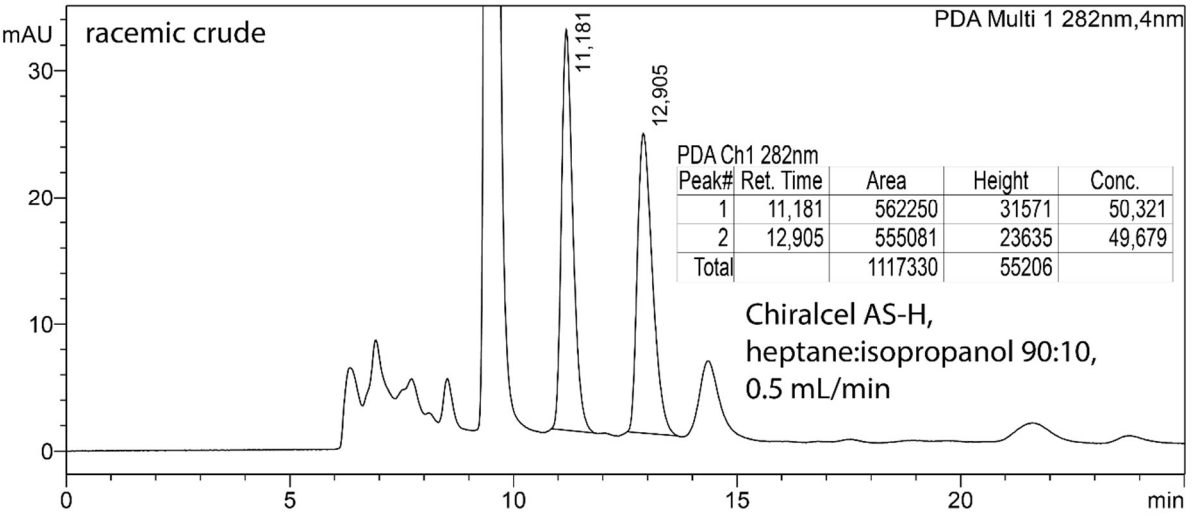
4-methyl-3-(2-methyl-1H-indol-3-yl)pentan-1-ol (3b)

Reference product and racemic crude product:

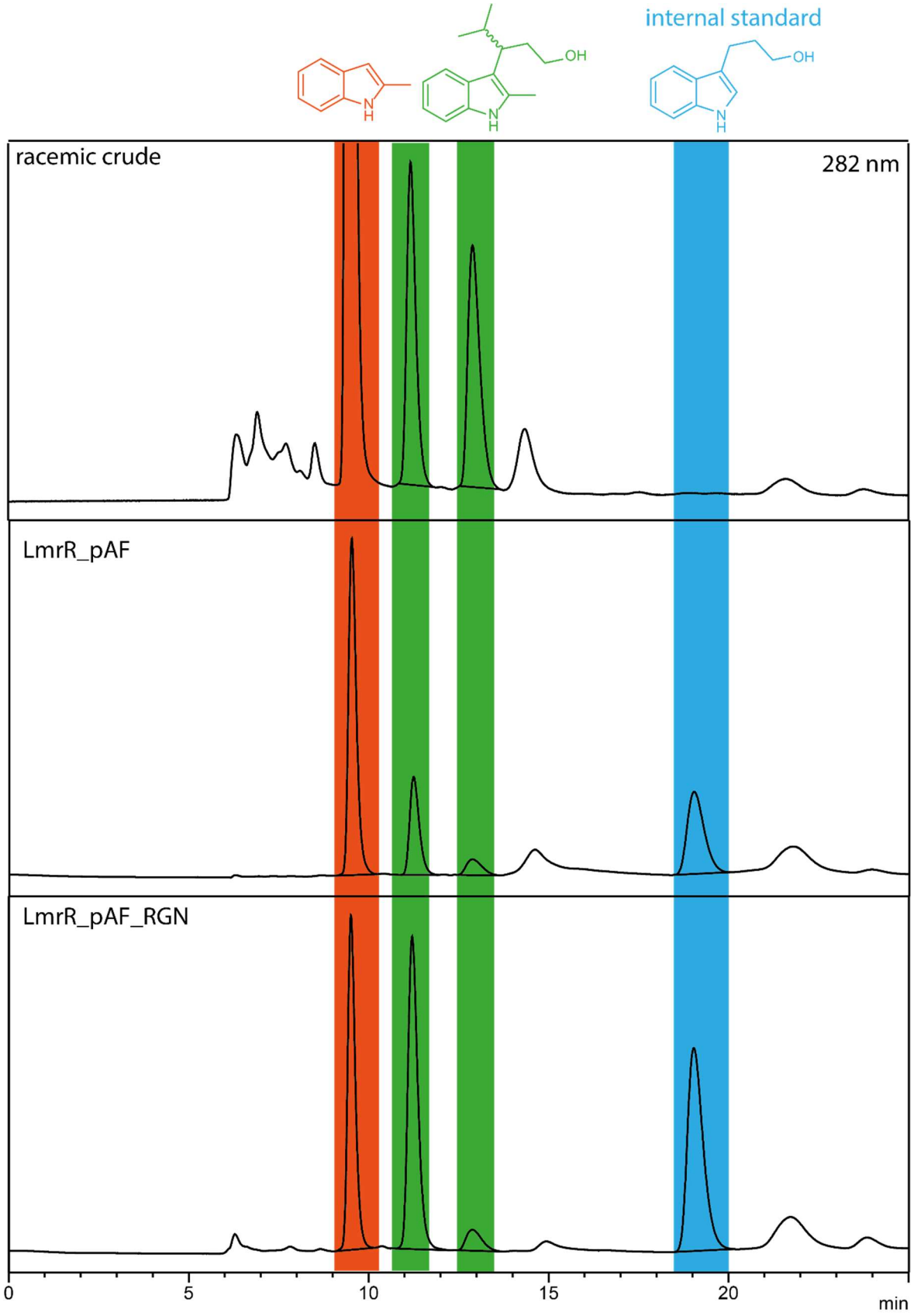
d





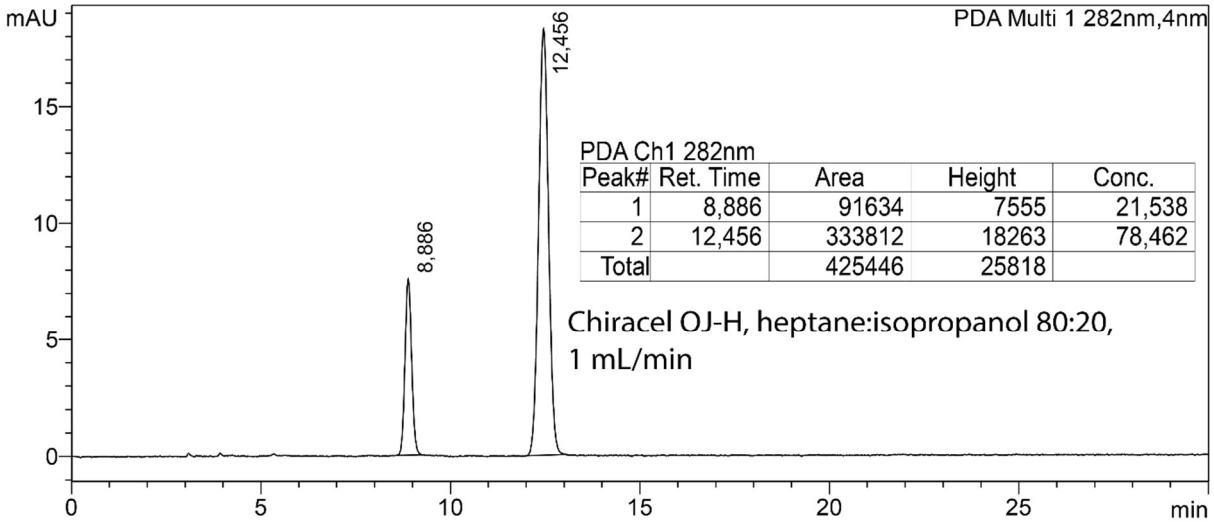


Artificial enzyme catalysed reactions: (Chiracel AS-H, heptane:isopropanol 90:10, 1 mL/min)



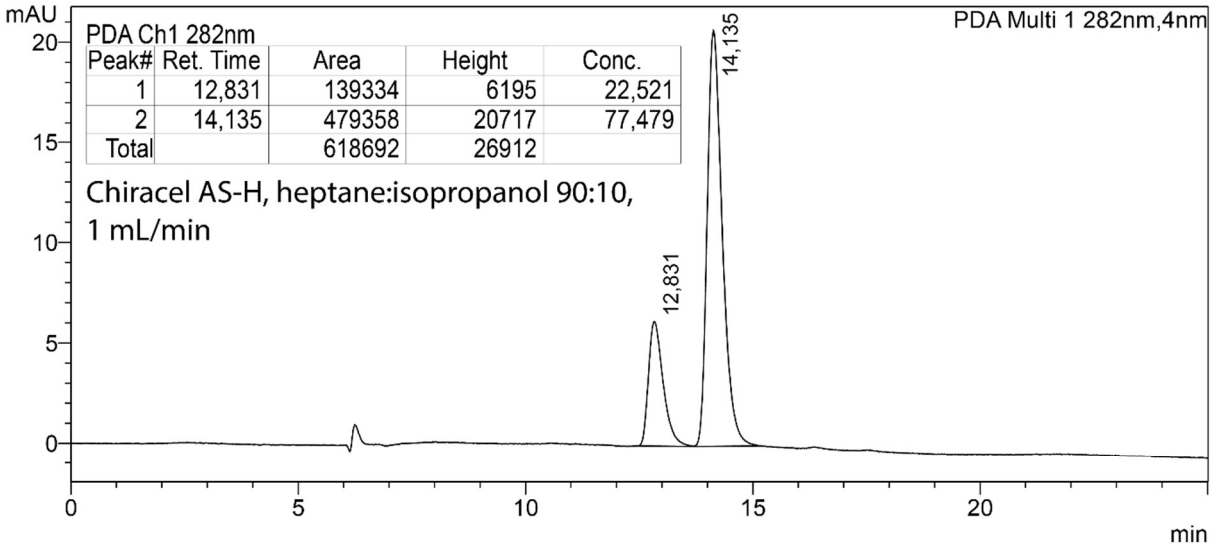
3-(2-methyl-1H-indol-3-yl)butan-1-ol (3c)

Reference compound and racemic crude product:



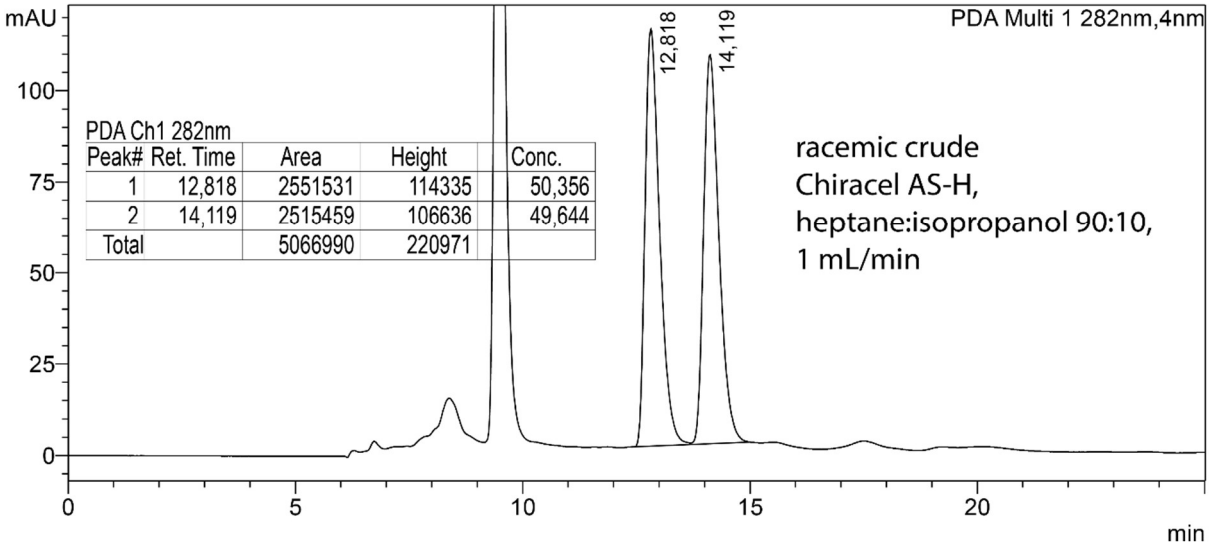
PDA Ch1 282nm

Peak#	Ret. Time	Area	Height	Conc.
1	8,886	91634	7555	21,538
2	12,456	333812	18263	78,462
Total		425446	25818	



PDA Ch1 282nm

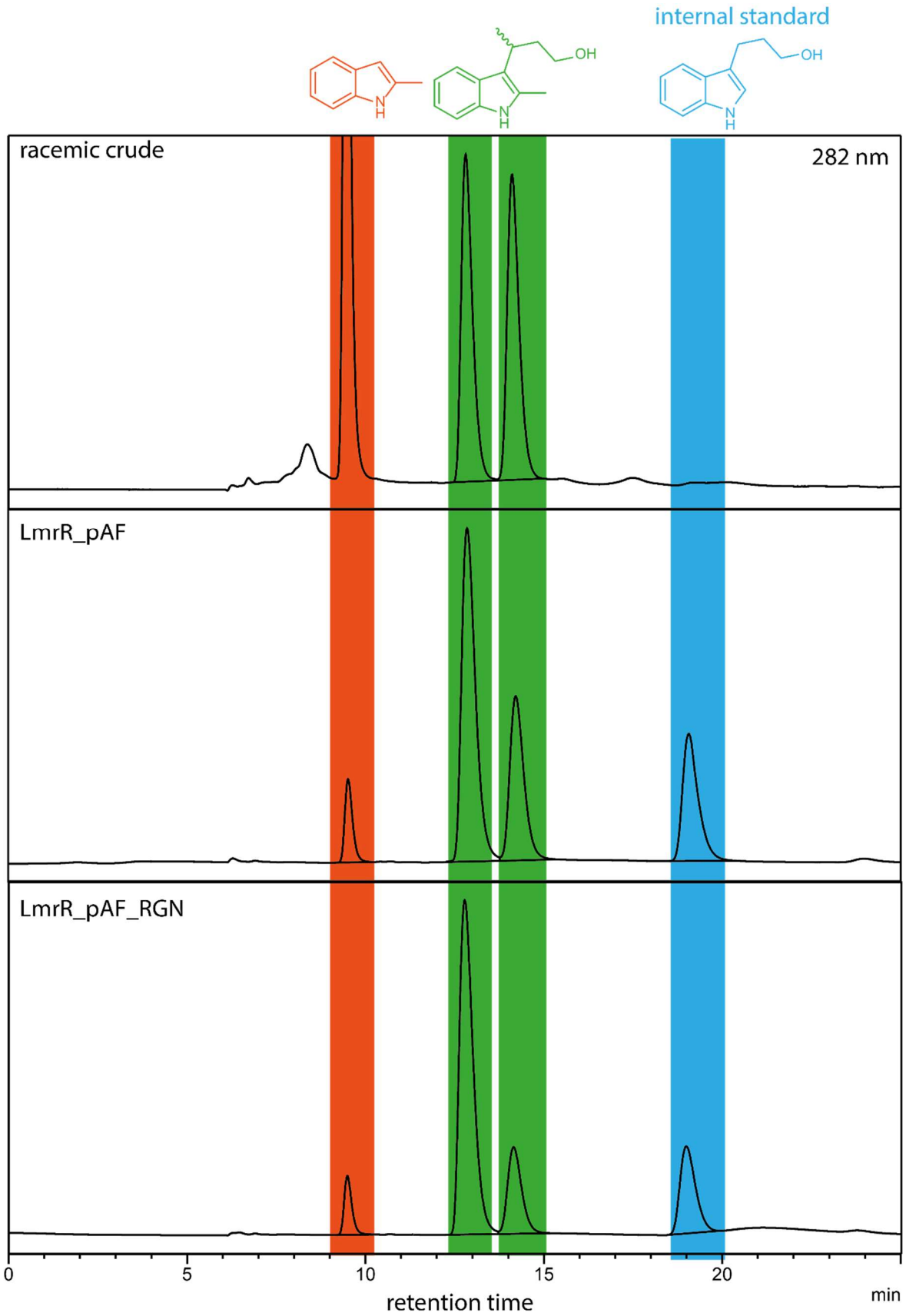
Peak#	Ret. Time	Area	Height	Conc.
1	12,831	139334	6195	22,521
2	14,135	479358	20717	77,479
Total		618692	26912	



PDA Ch1 282nm

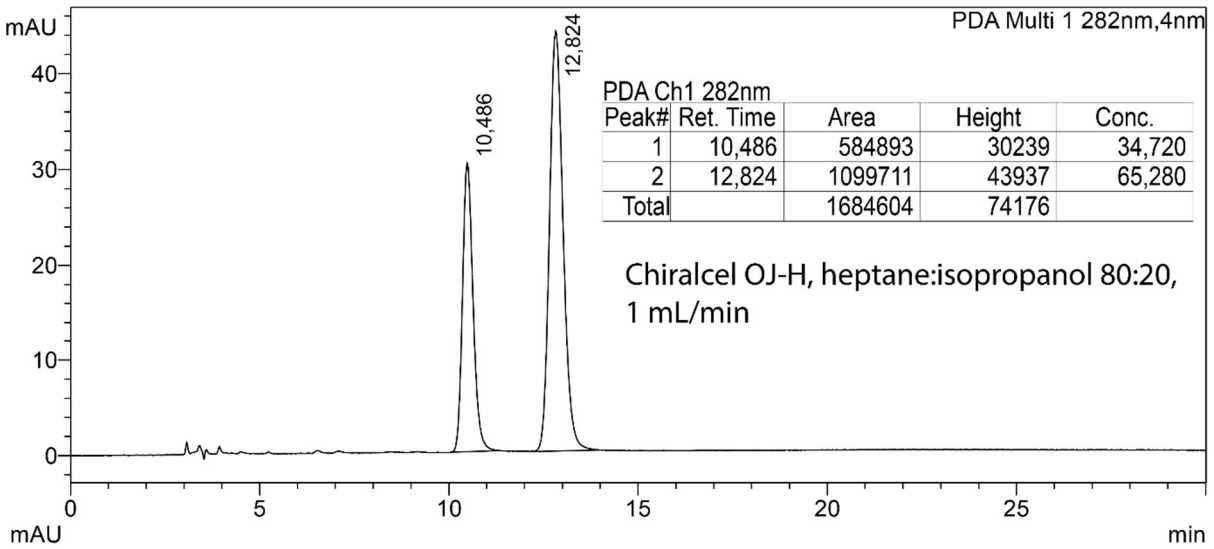
Peak#	Ret. Time	Area	Height	Conc.
1	12,818	2551531	114335	50,356
2	14,119	2515459	106636	49,644
Total		5066990	220971	

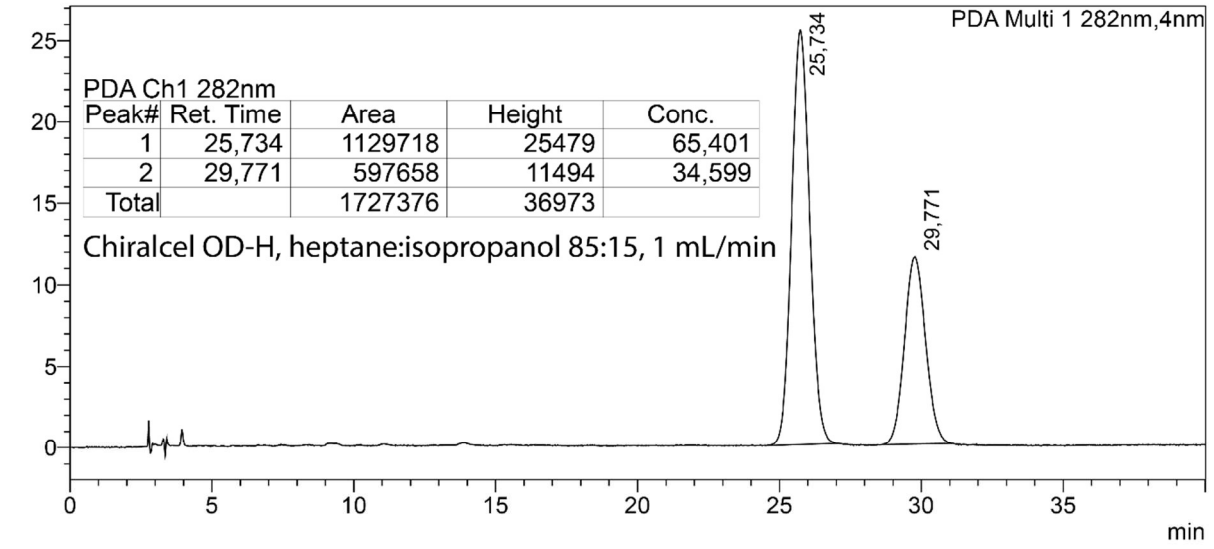
Artificial enzyme catalyzed reaction: (Chiracel AS-H, heptane:isopropanol 90:10, 0.5 mL/min)

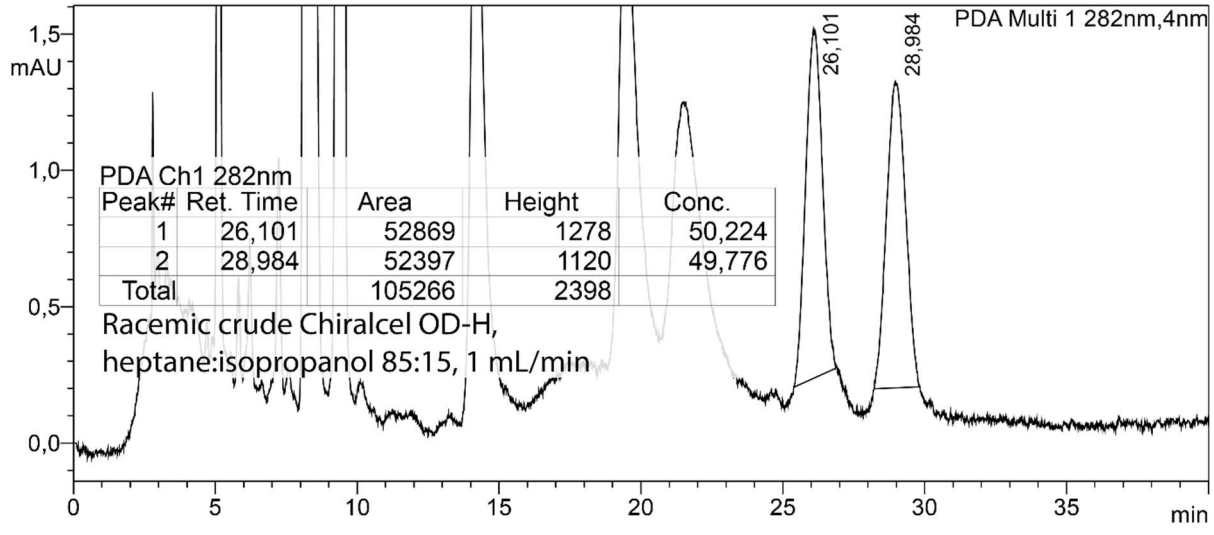


3-(2-methyl-1H-indol-3-yl)-3-phenylpropan-1-ol (3d)

Reference compound and racemic crude product:

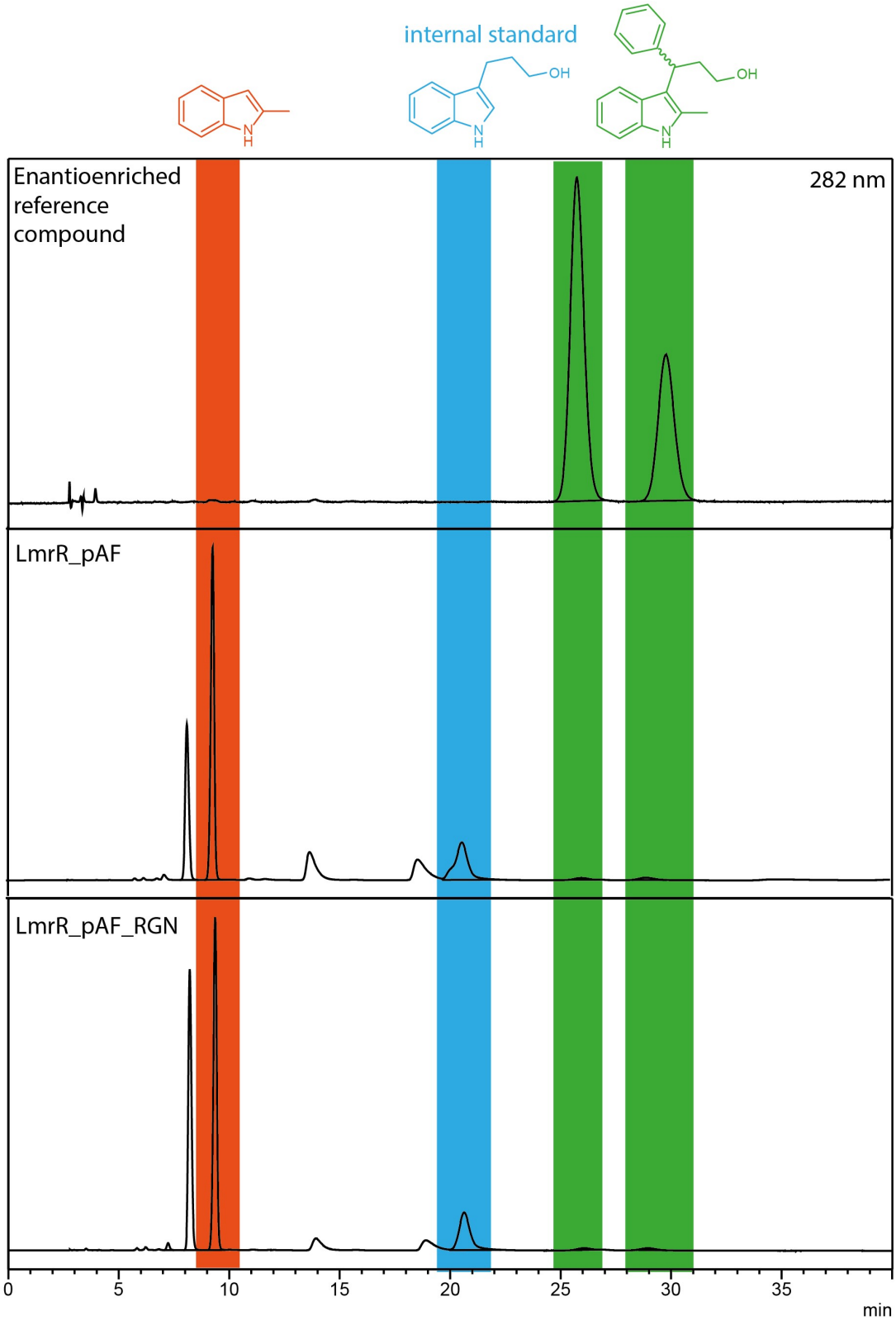






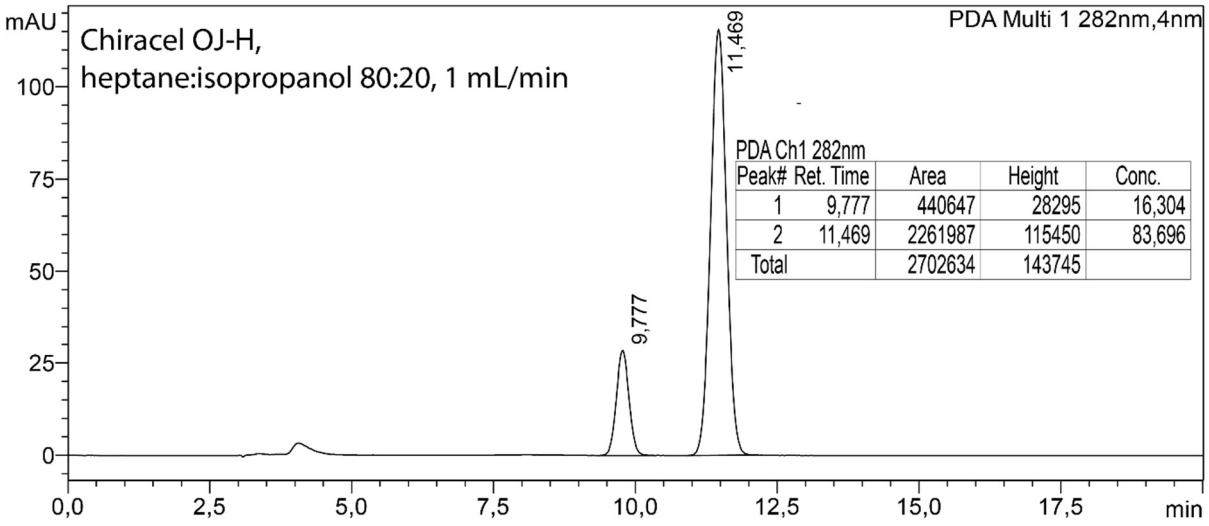
Artificial enzyme catalysed reaction: (Chiracel OD-H, heptane:isopropanol 85:15, 1 mL/min)

On account of abundant side-products evident in the racemic reaction, the enantioenriched reference compound chromatogram is shown instead for comparison.



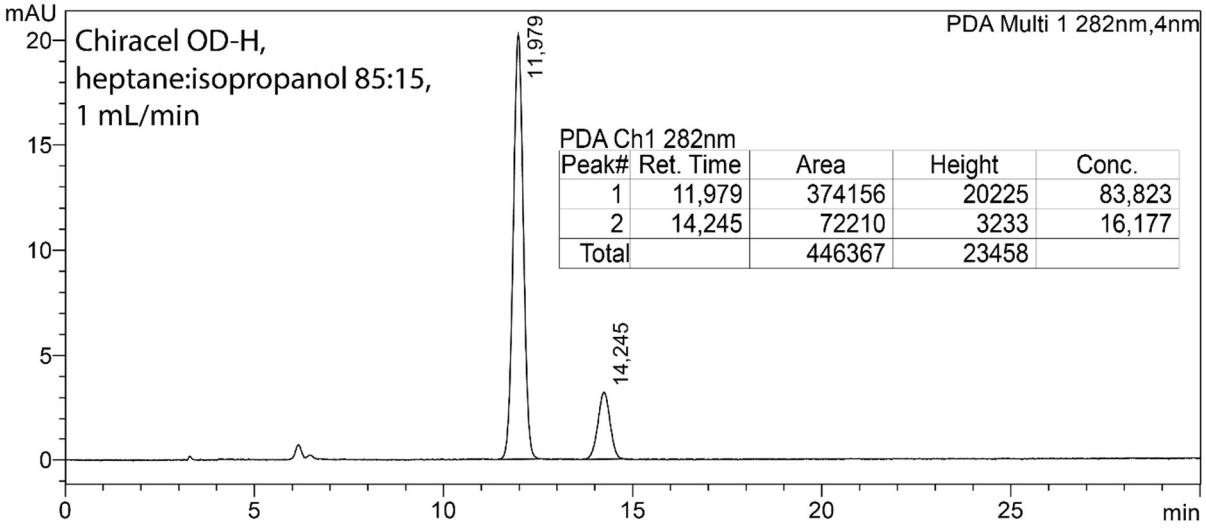
3-(1H-indol-3-yl)hexan-1-ol (3e)

Reference compound and racemic crude:



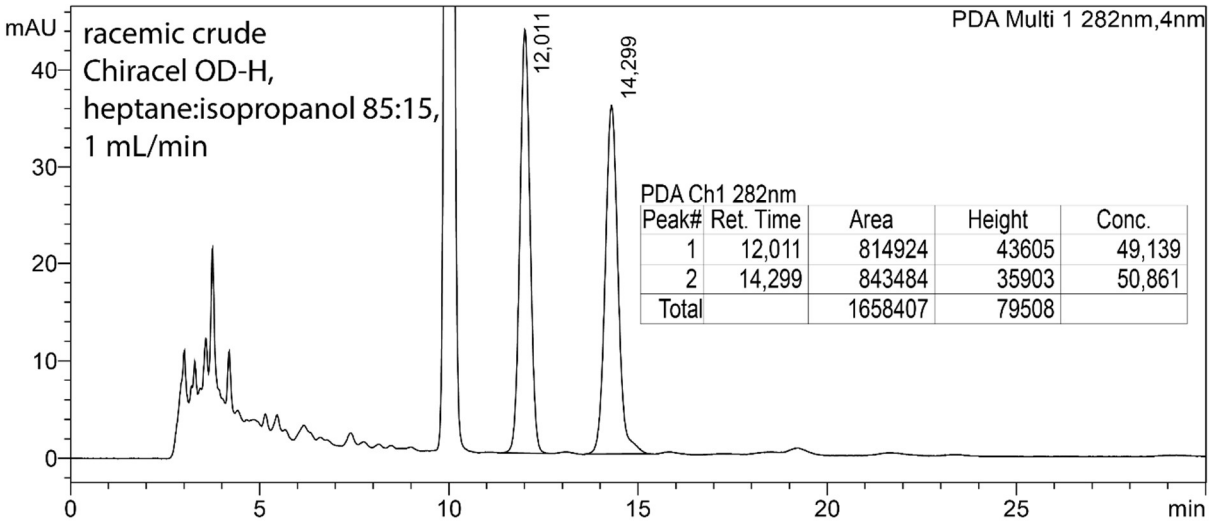
PDA Ch1 282nm

Peak#	Ret. Time	Area	Height	Conc.
1	9,777	440647	28295	16,304
2	11,469	2261987	115450	83,696
Total		2702634	143745	



PDA Ch1 282nm

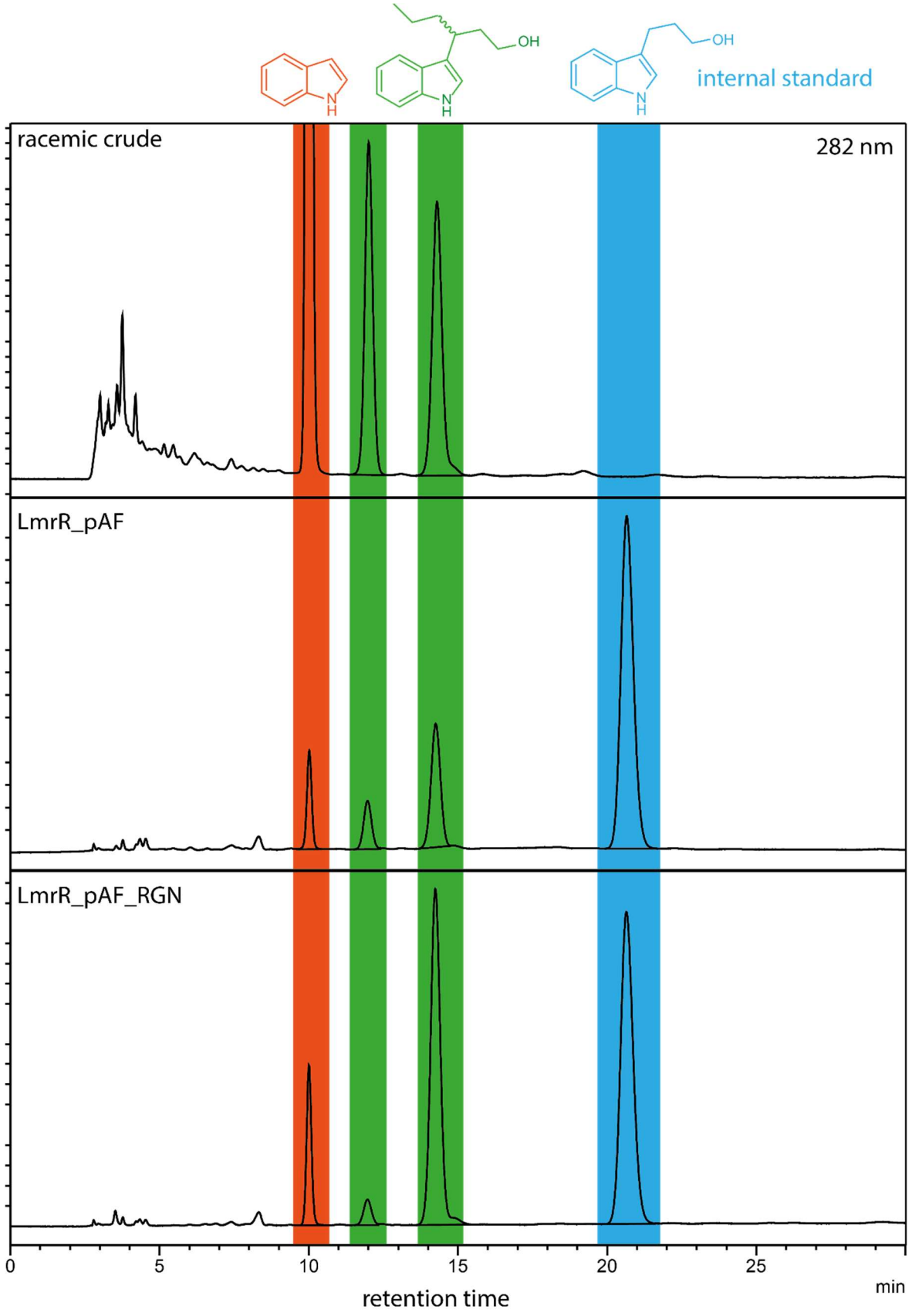
Peak#	Ret. Time	Area	Height	Conc.
1	11,979	374156	20225	83,823
2	14,245	72210	3233	16,177
Total		446367	23458	



PDA Ch1 282nm

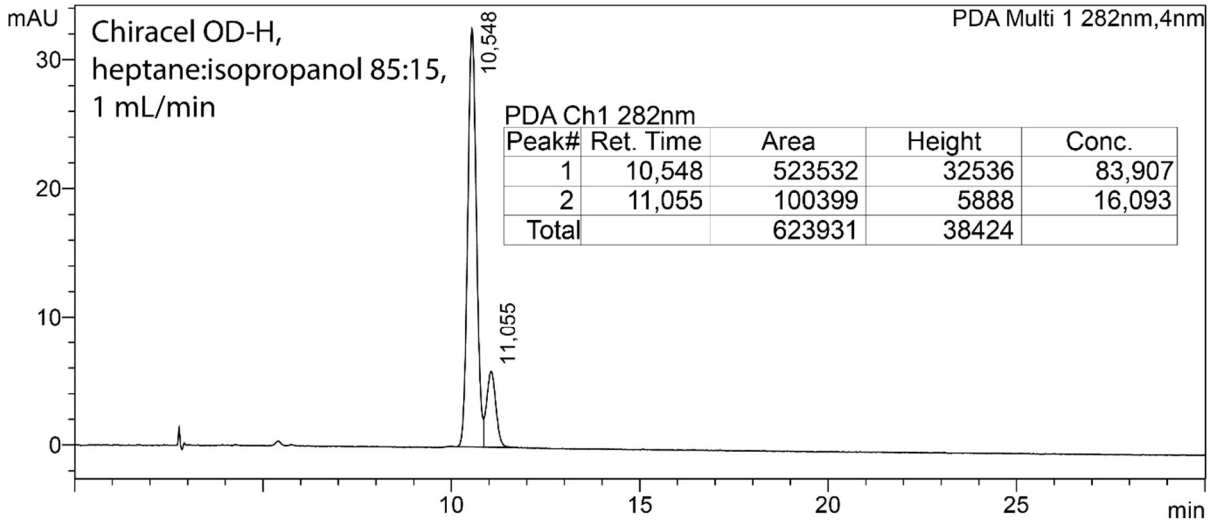
Peak#	Ret. Time	Area	Height	Conc.
1	12,011	814924	43605	49,139
2	14,299	843484	35903	50,861
Total		1658407	79508	

Artificial enzyme catalysed reaction: (Chiracel OD-H, heptane:isopropanol 85:15, 1 mL/min)



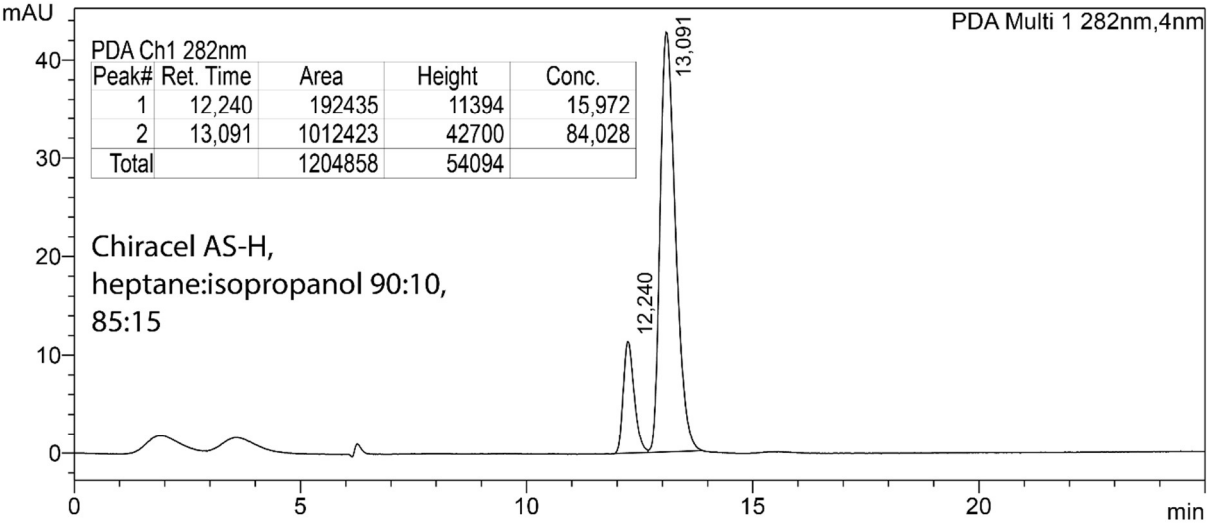
3-(5-methyl-1H-indol-3-yl)hexan-1-ol (3f)

Reference compound and racemic crude:



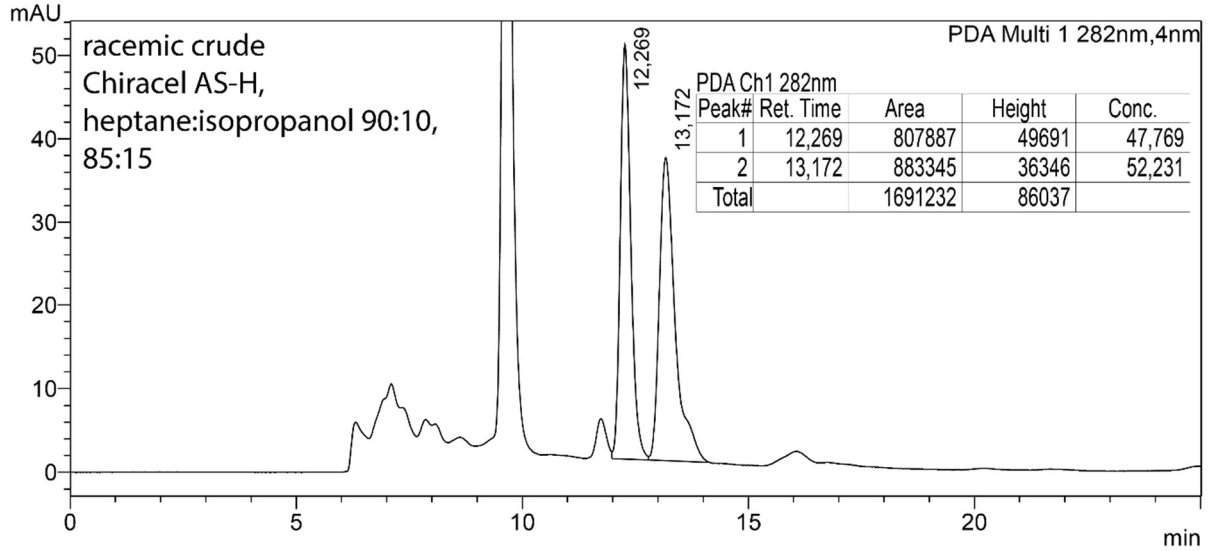
PDA Ch1 282nm

Peak#	Ret. Time	Area	Height	Conc.
1	10,548	523532	32536	83,907
2	11,055	100399	5888	16,093
Total		623931	38424	



PDA Ch1 282nm

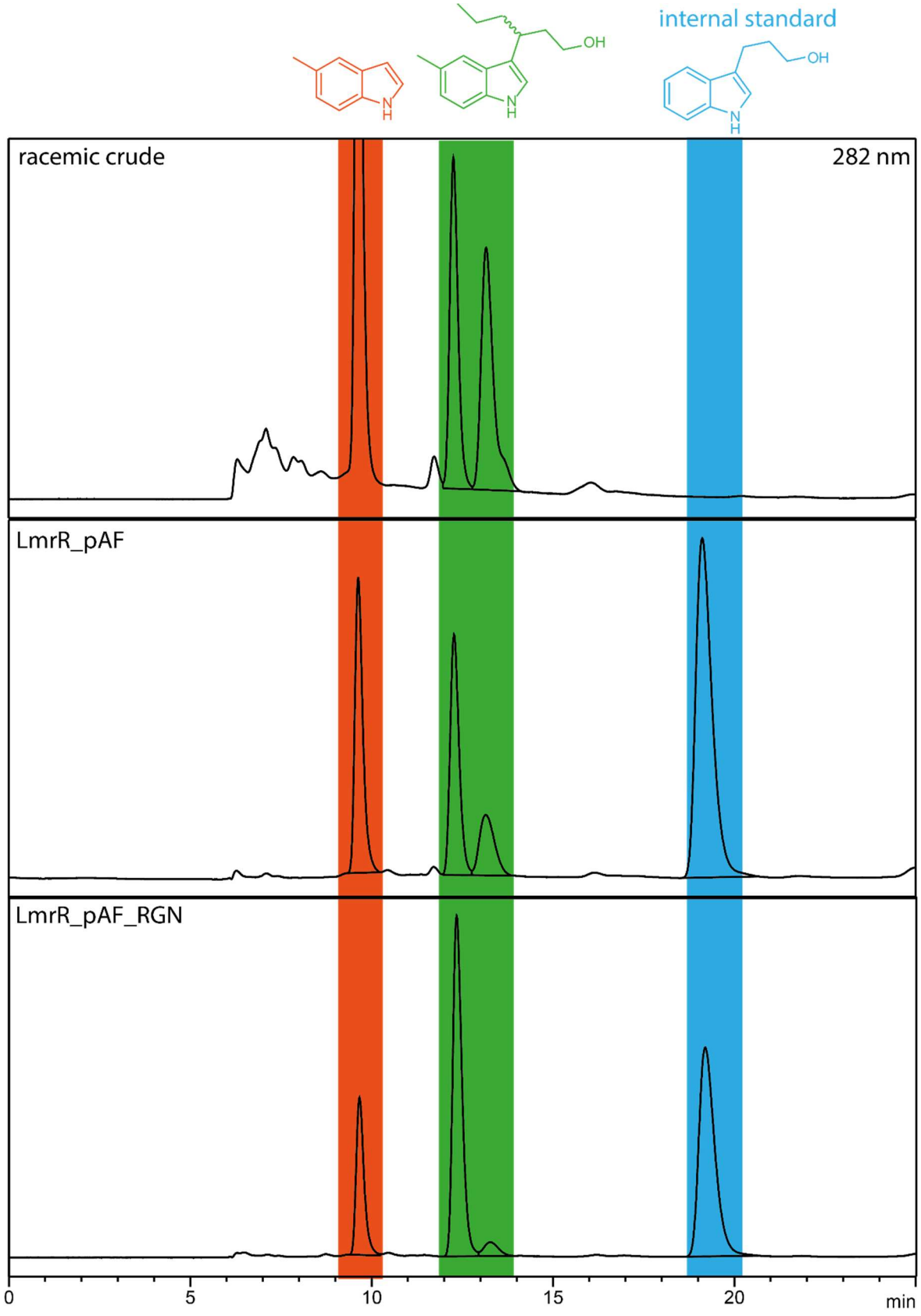
Peak#	Ret. Time	Area	Height	Conc.
1	12,240	192435	11394	15,972
2	13,091	1012423	42700	84,028
Total		1204858	54094	



PDA Ch1 282nm

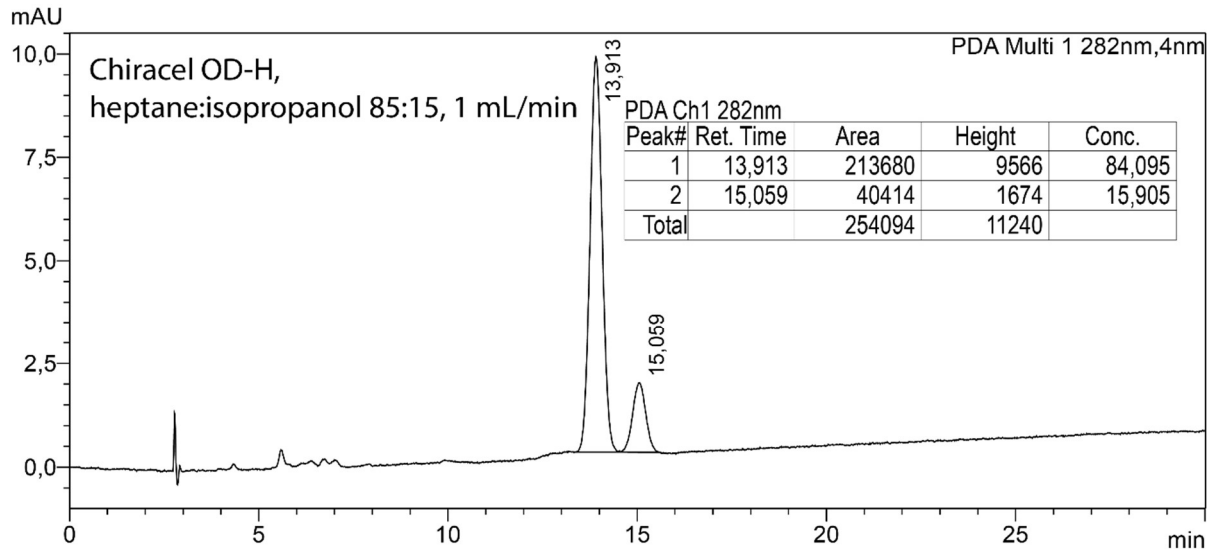
Peak#	Ret. Time	Area	Height	Conc.
1	12,269	807887	49691	47,769
2	13,172	883345	36346	52,231
Total		1691232	86037	

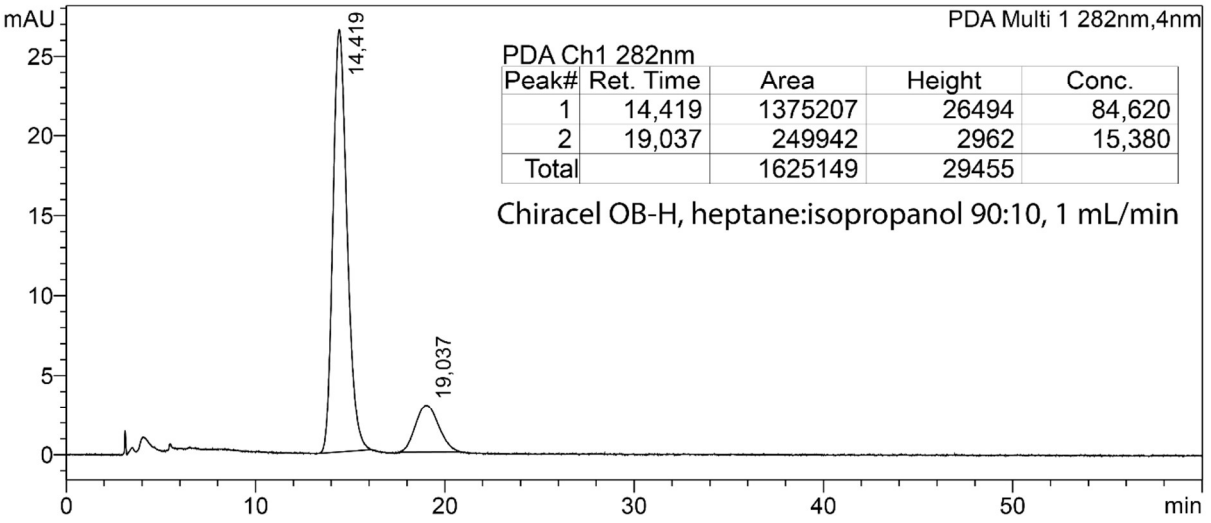
Artificial enzyme catalysed reaction: (Chiracel AS-H, heptane:isopropanol 90:10, 0.5 mL/min)

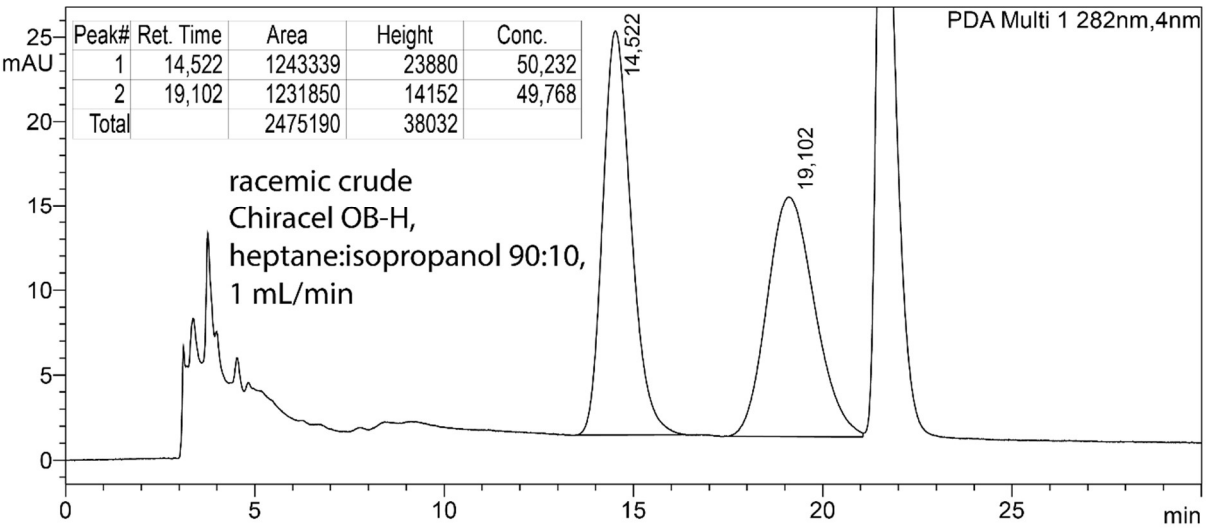


3-(5-methoxy-1H-indol-3-yl)hexan-1-ol (3g)

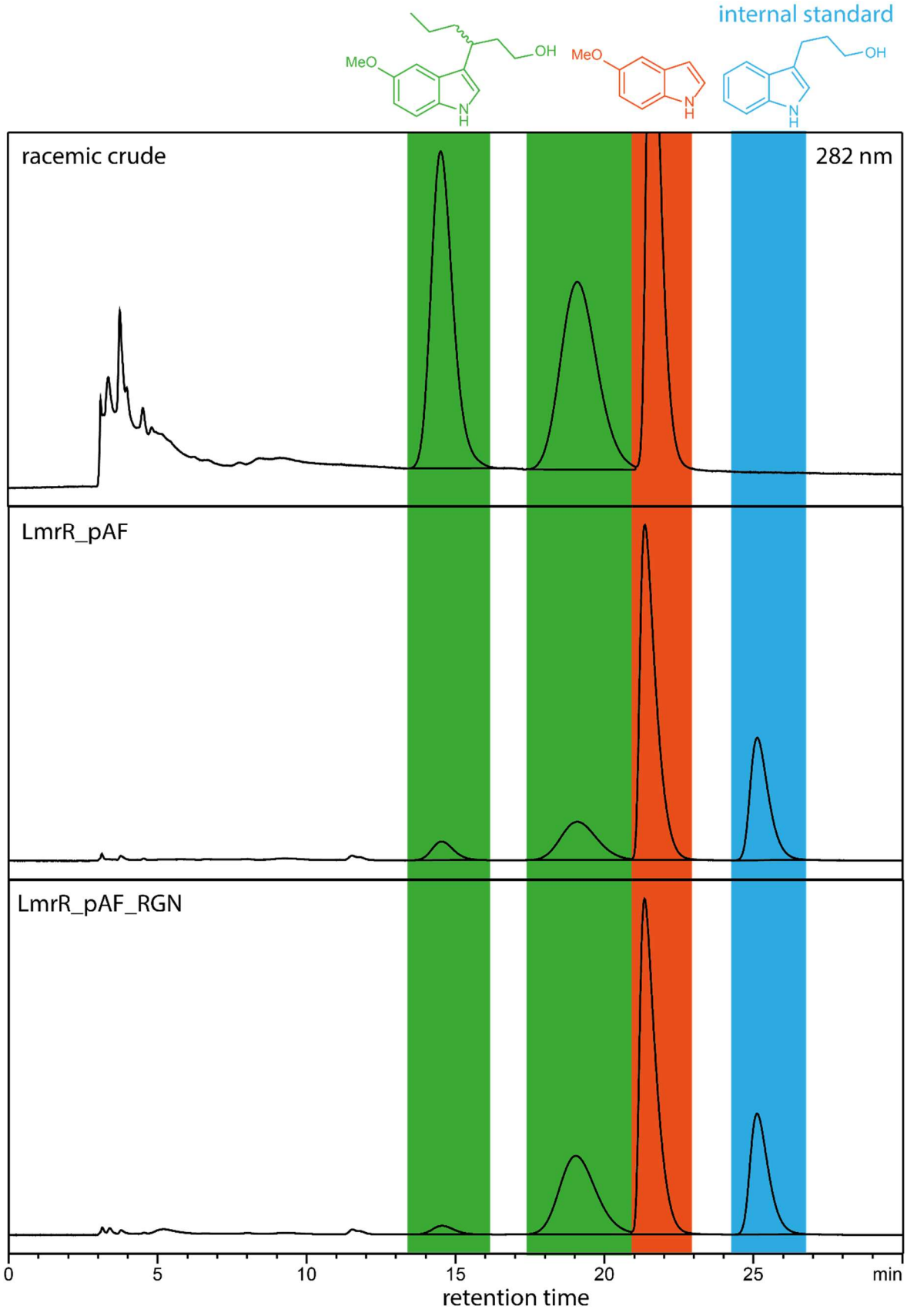
Reference compound and racemic crude:





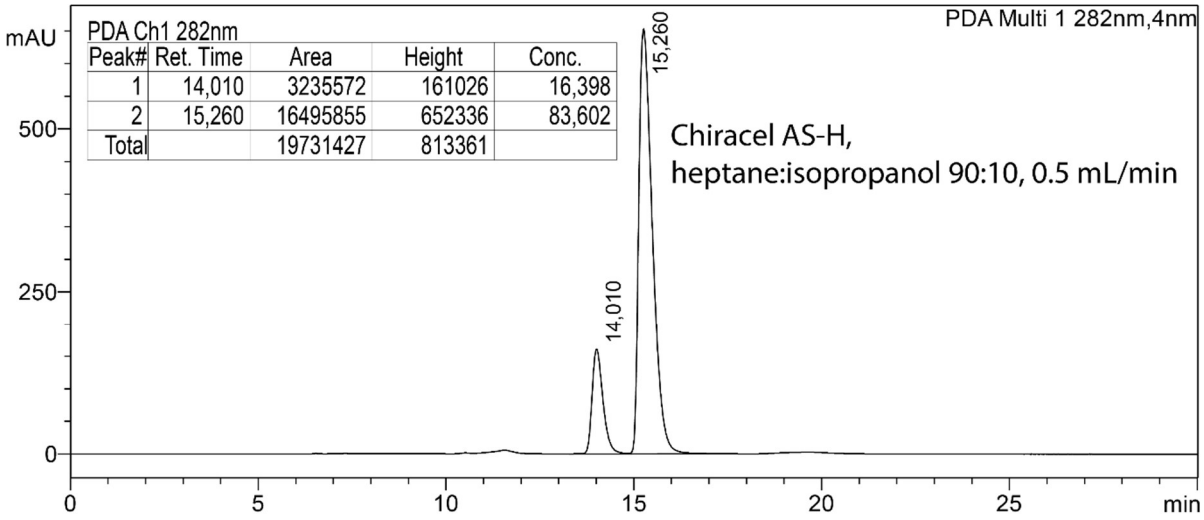


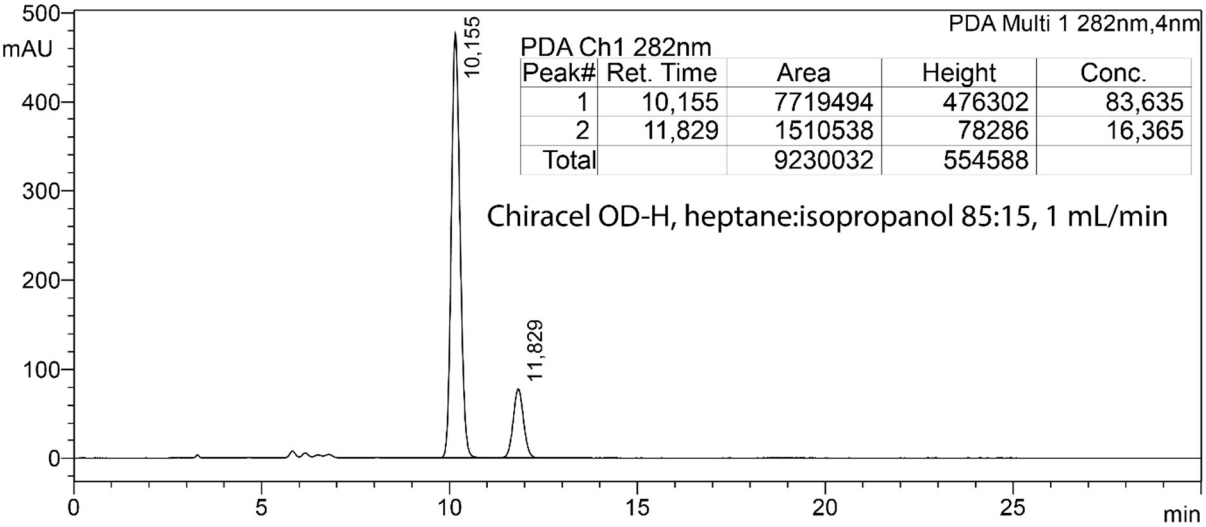
Artificial enzyme catalysed reaction: (Chiracel OB-H, heptane:isopropanol 90:10, 1 mL/min)

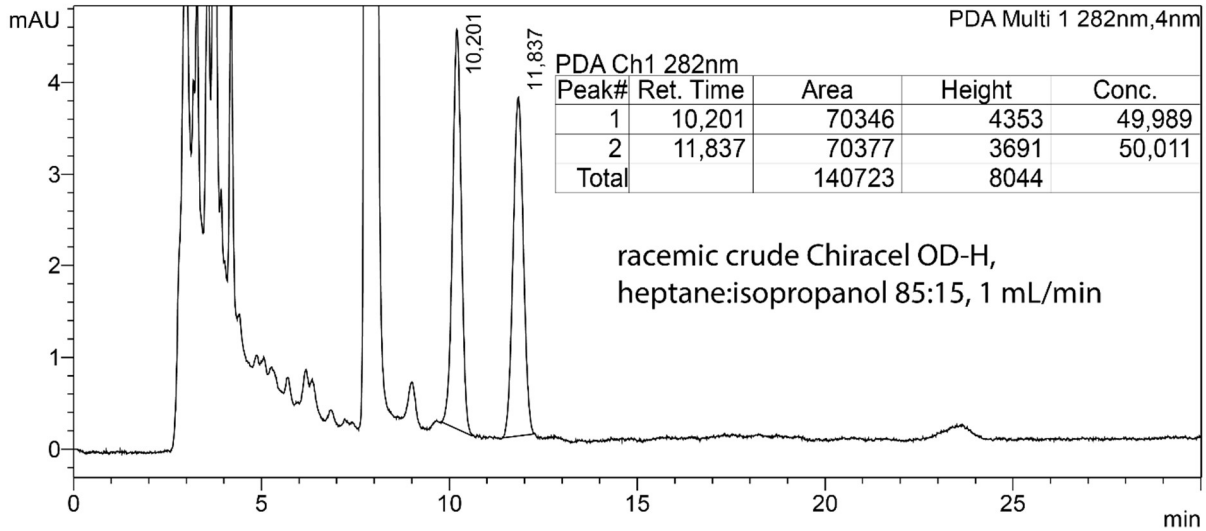


3-(5-bromo-1H-indol-3-yl)hexan-1-ol (3h)

Reference compound and racemic crude:

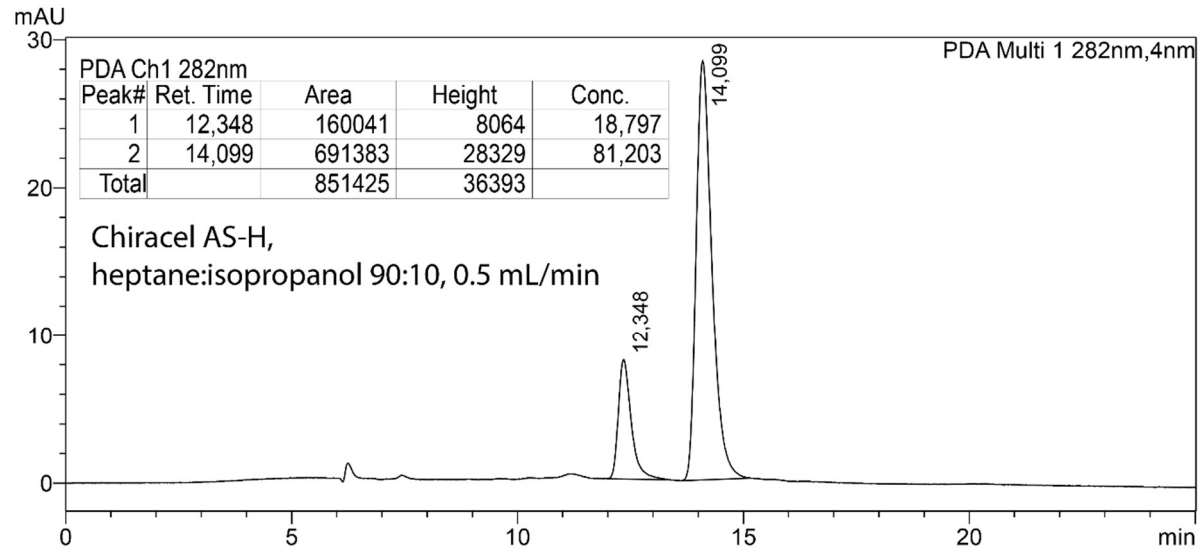


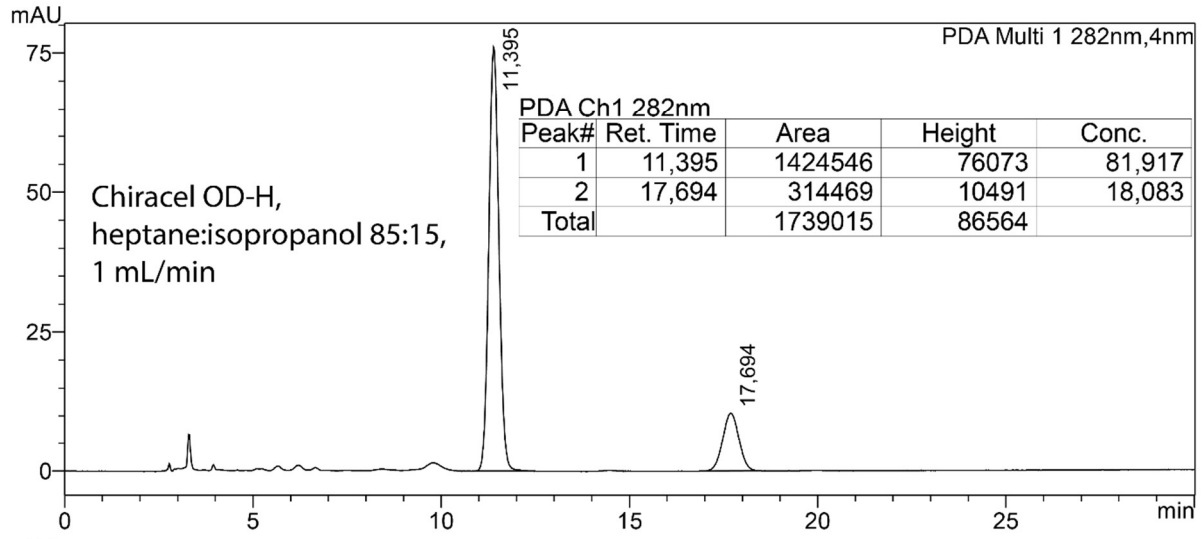


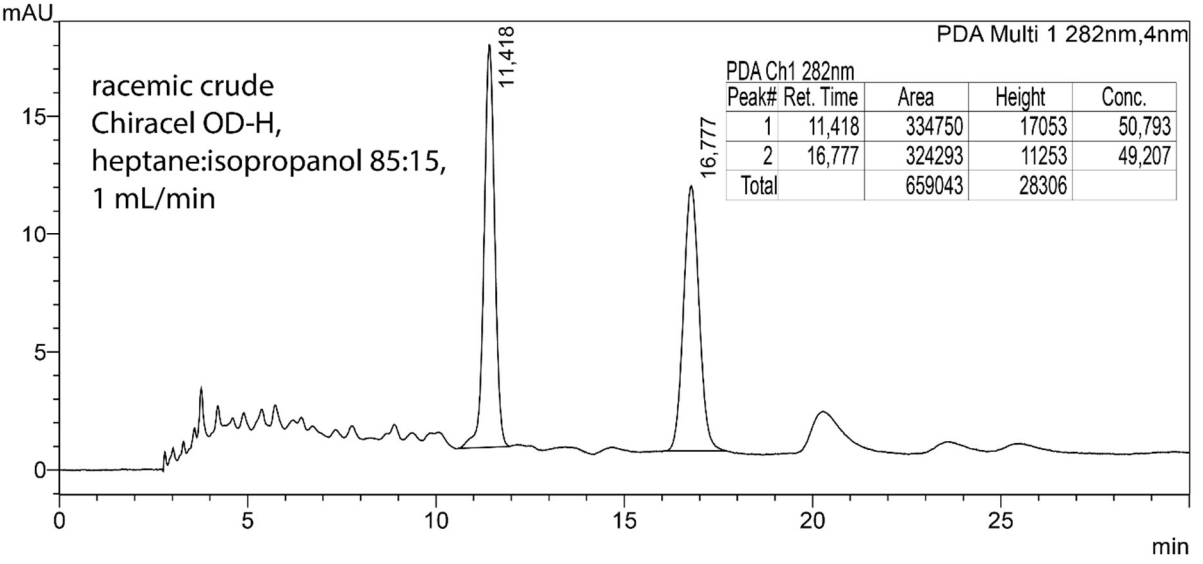


3-(5-methoxy-2-methyl-1H-indol-3-yl)hexan-1-ol (3i)

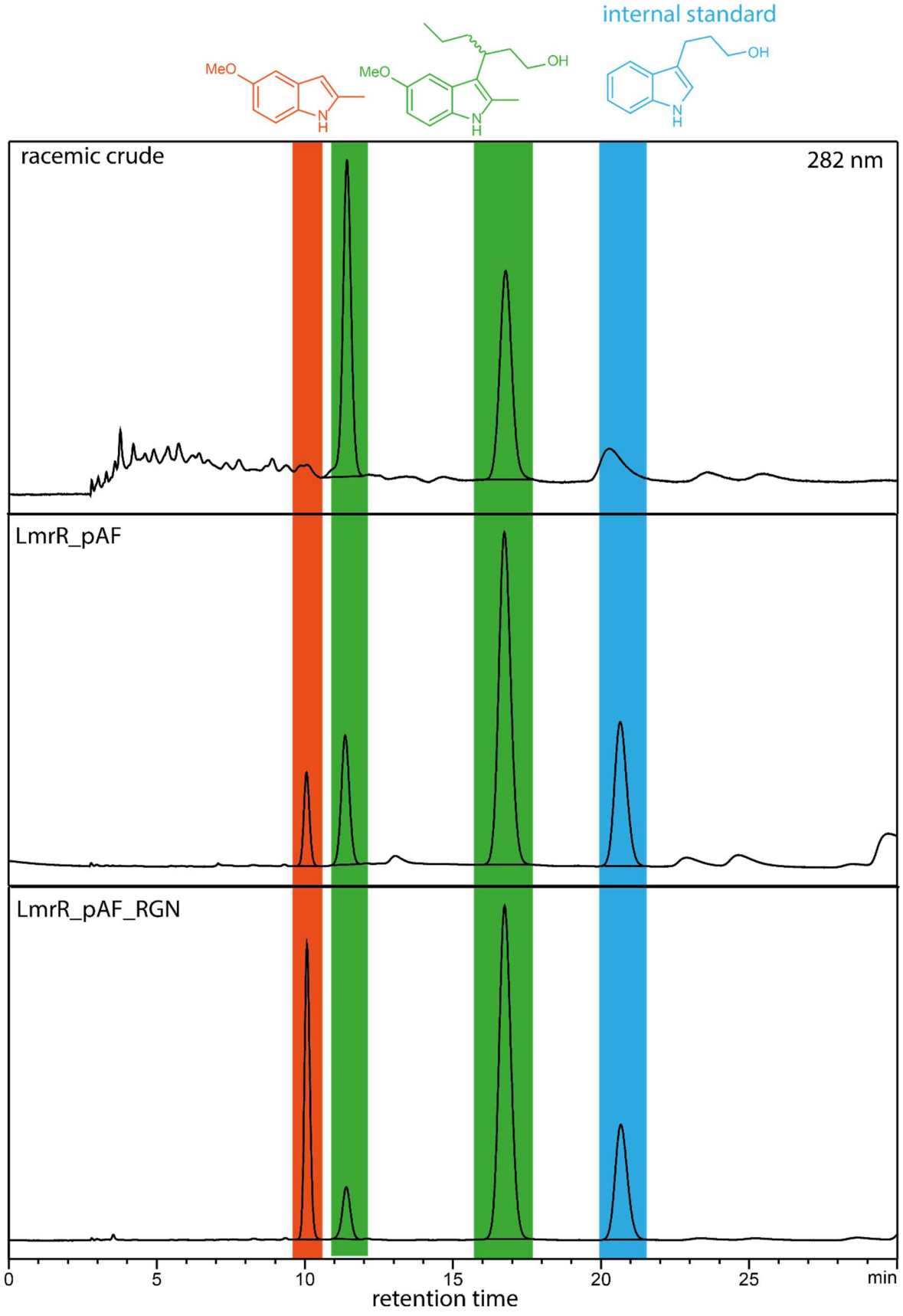
Reference compound and racemic crude:



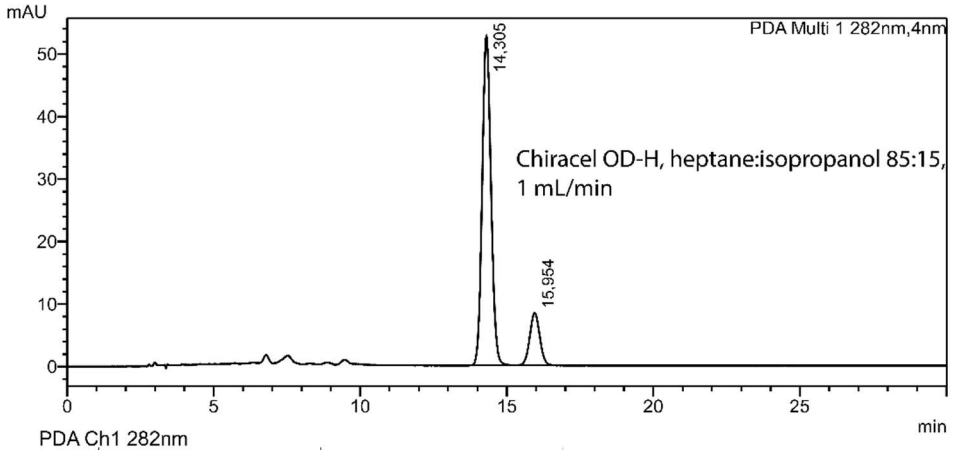




Artificial enzyme catalysed reactions: (Chiracel OD-H, heptane:isopropanol 85:15, 1 mL/min)

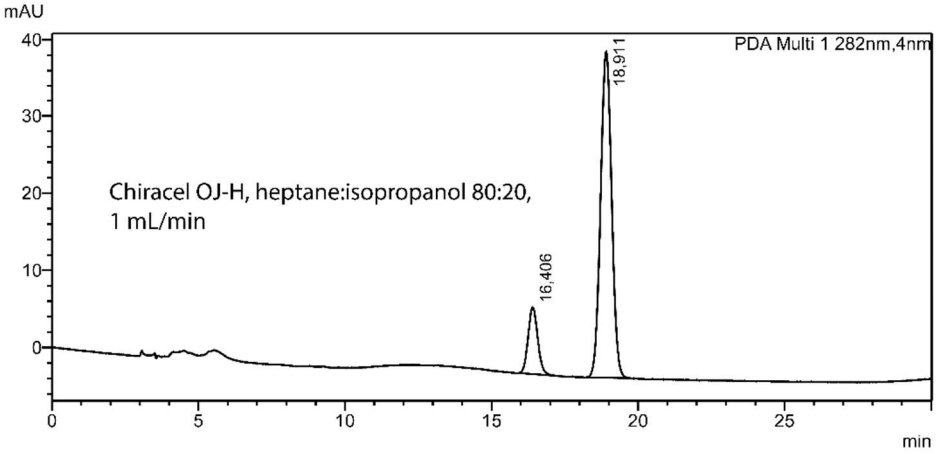


3-(1H-indol-3-yl)butan-1-ol (3j)



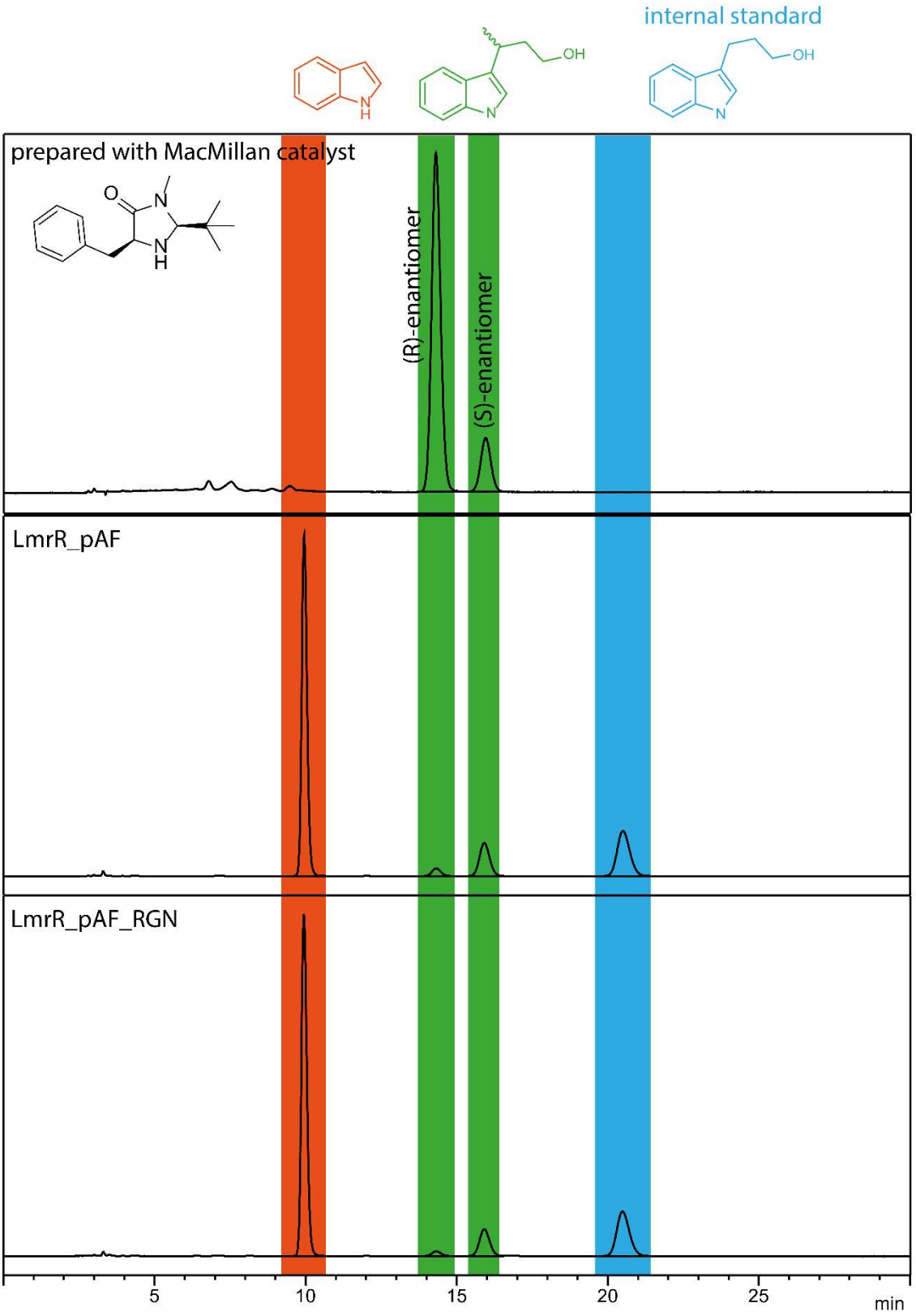
PDA Ch1 282nm

Peak#	Ret. Time	Area	Height	Conc.
1	14,305	1105386	52589	84,79
2	15,954	198311	8368	15,21
Total		1303696	60957	



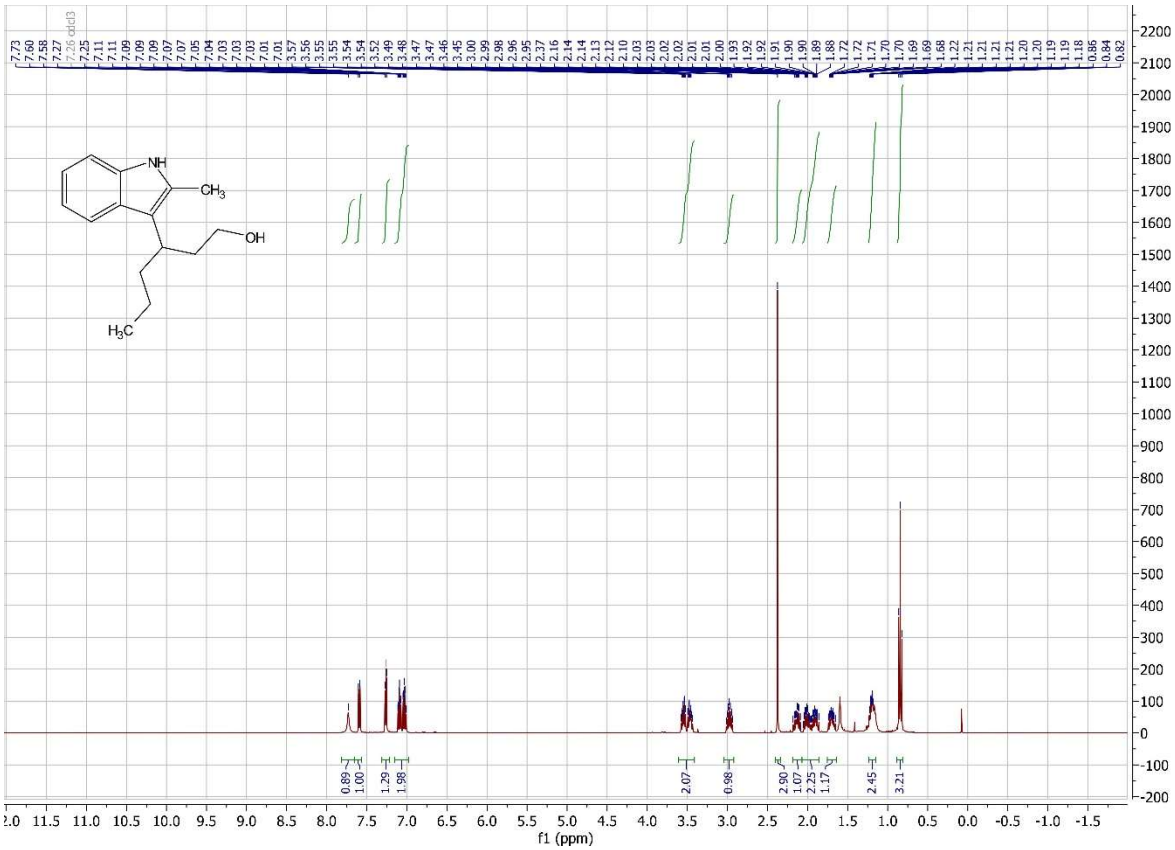
PDA Ch1 282nm

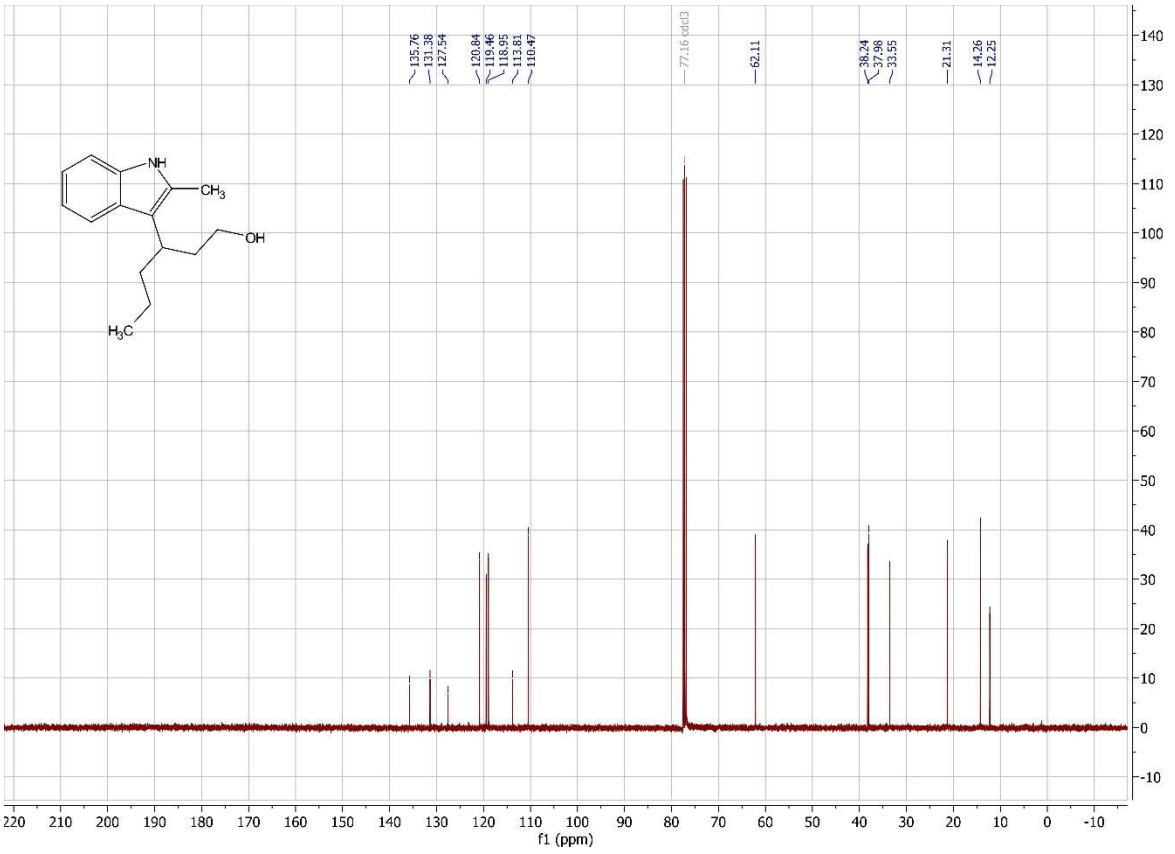
Peak#	Ret. Time	Area	Height	Conc.
1	16,406	198111	8642	15,12
2	18,911	1112020	42311	84,88
Total		1310131	50953	


internal standard
OH
OH
prepared with MacMillan catalyst
O
N
N
H
(R)-enantiomer
(S)-enantiomer
LmrR_pAF
LmrR_pAF_RGN
5
10
15
20
25
min

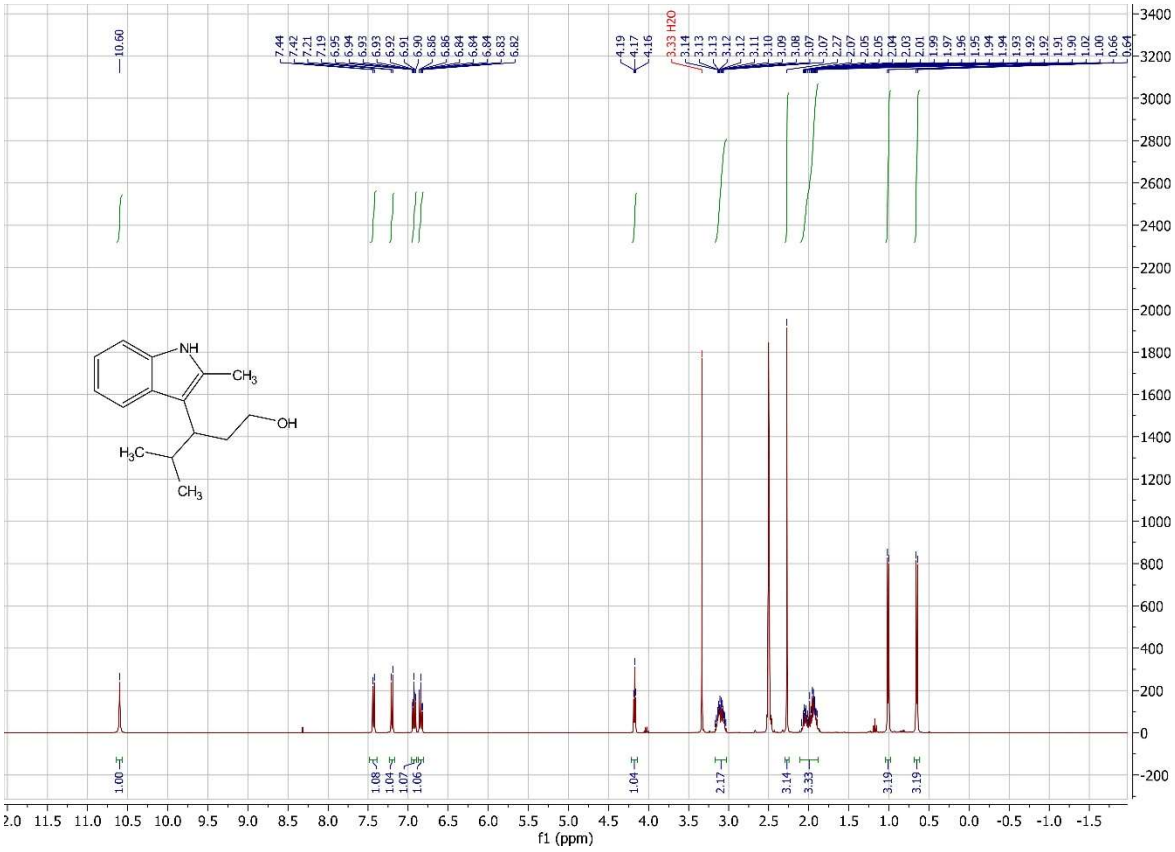
10. NMR Spectra

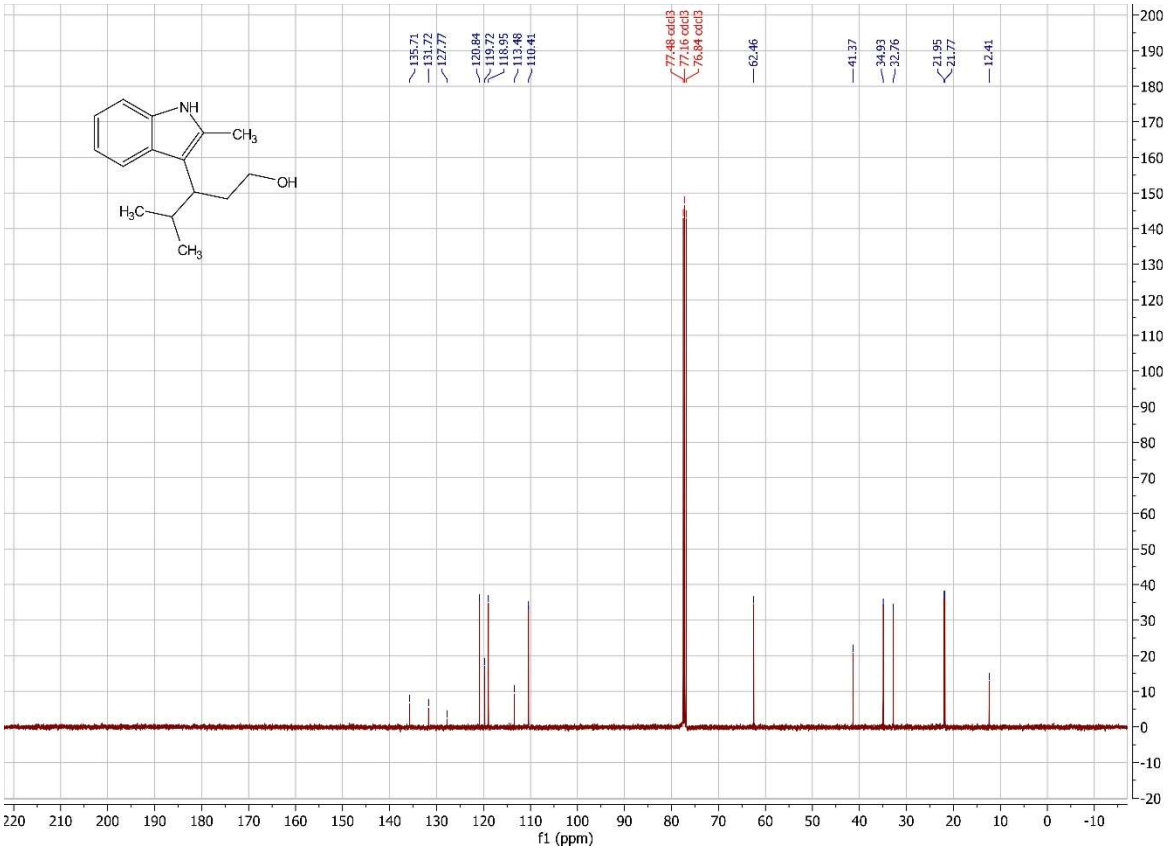
3-(2-methyl-1H-indol-3-yl)hexan-1-ol (3a)



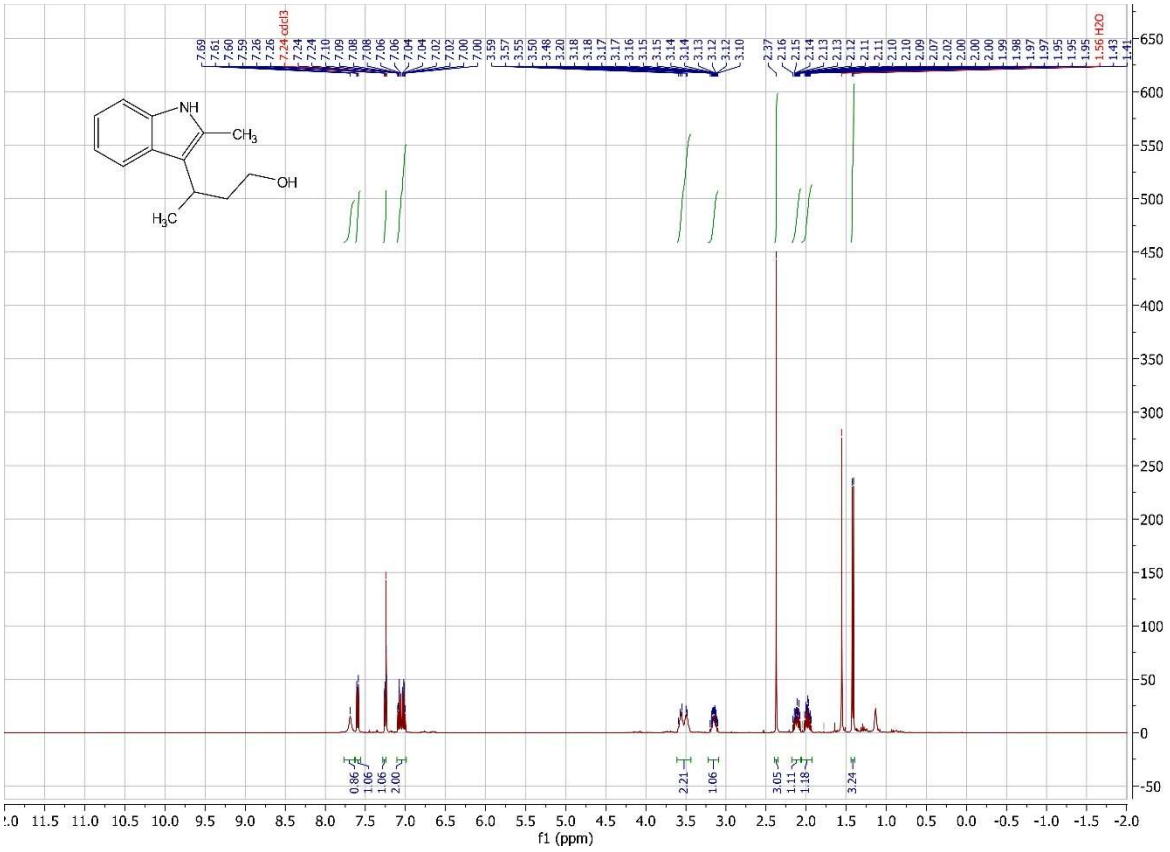


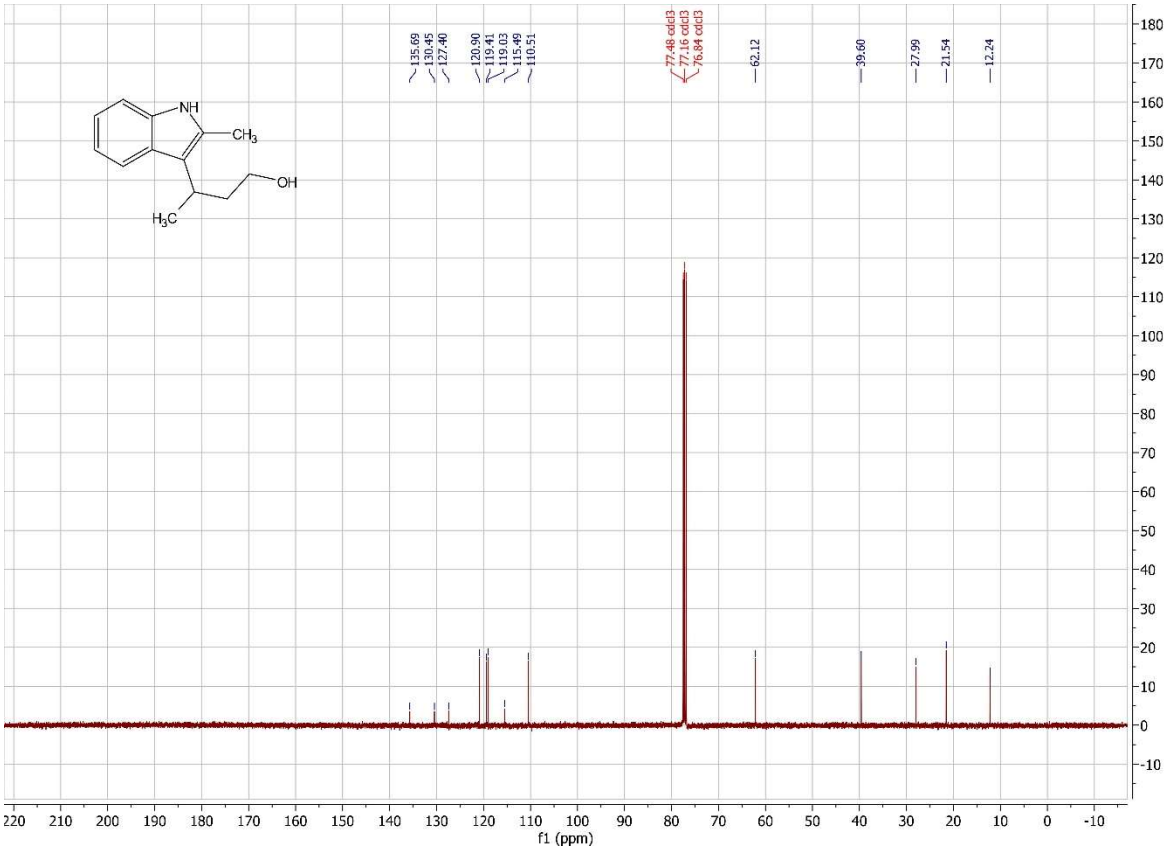
4-methyl-3-(2-methyl-1H-indol-3-yl)pentan-1-ol (3b)



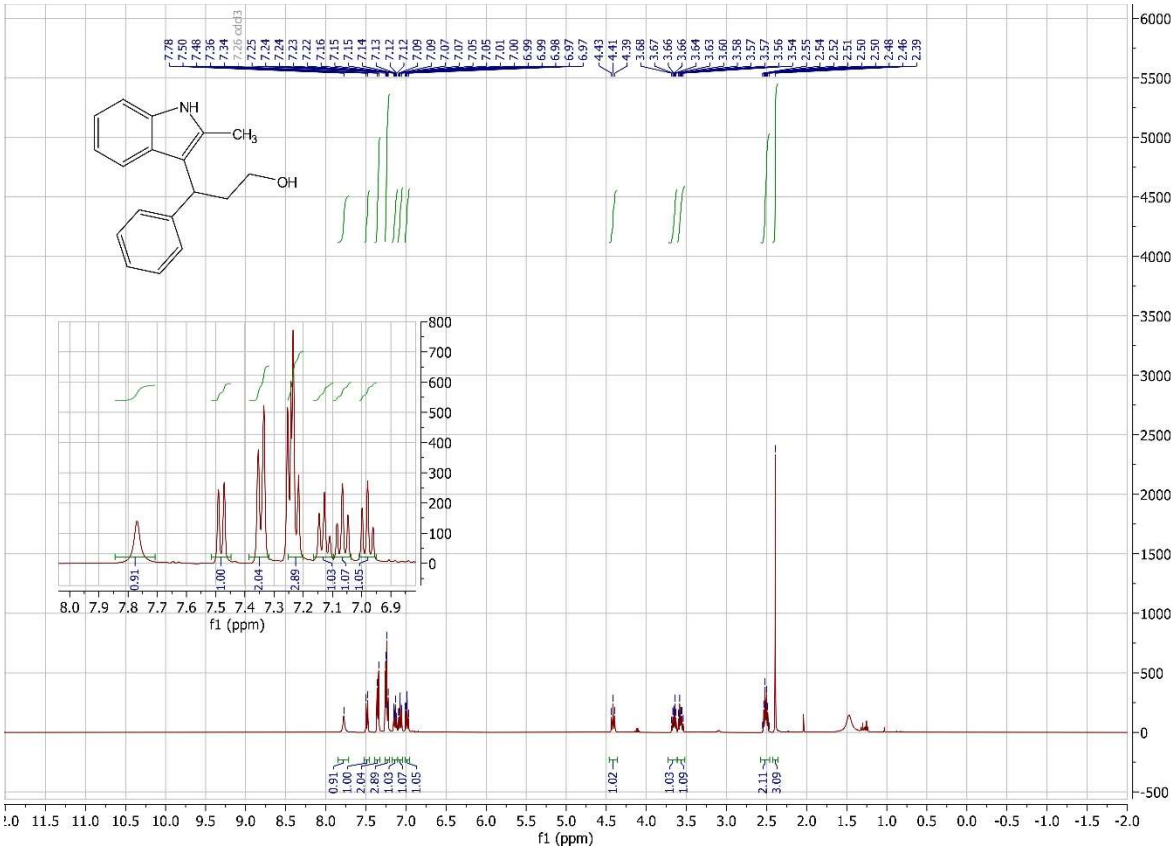


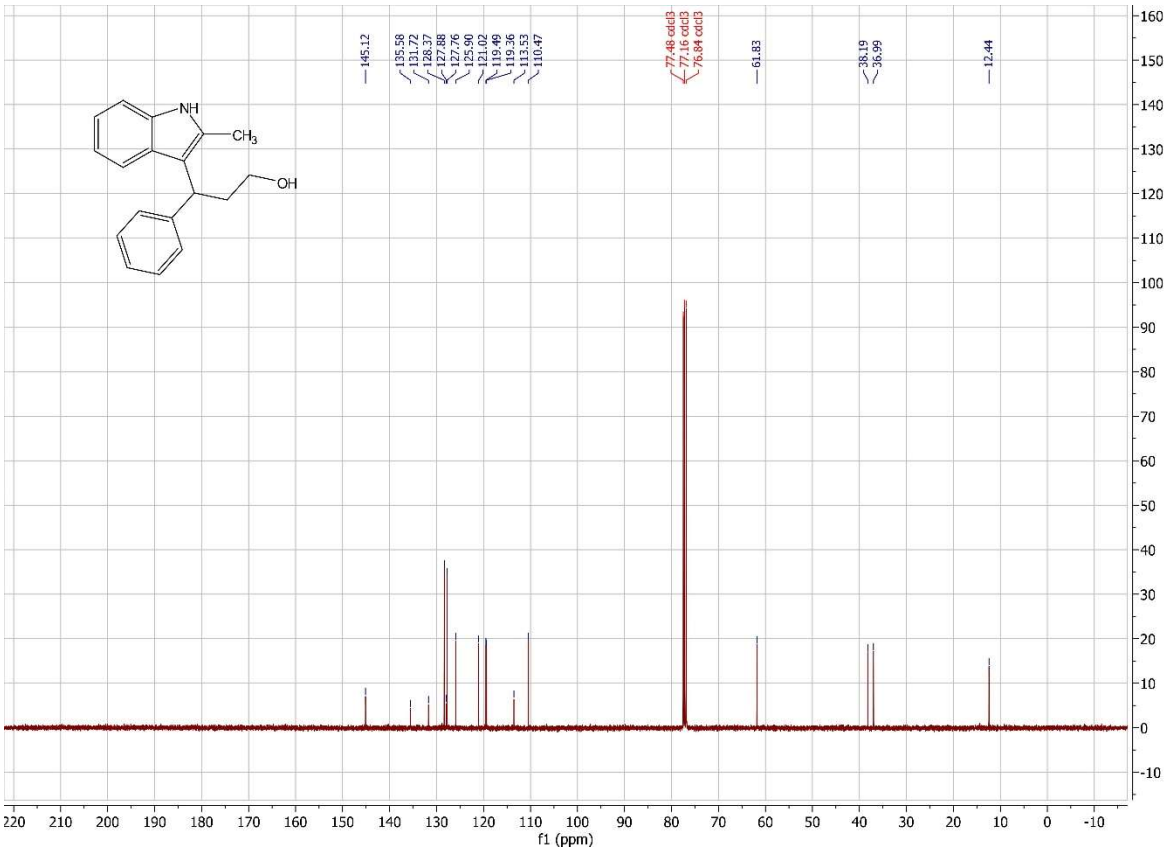
3-(2-methyl-1H-indol-3-yl)butan-1-ol (3c)



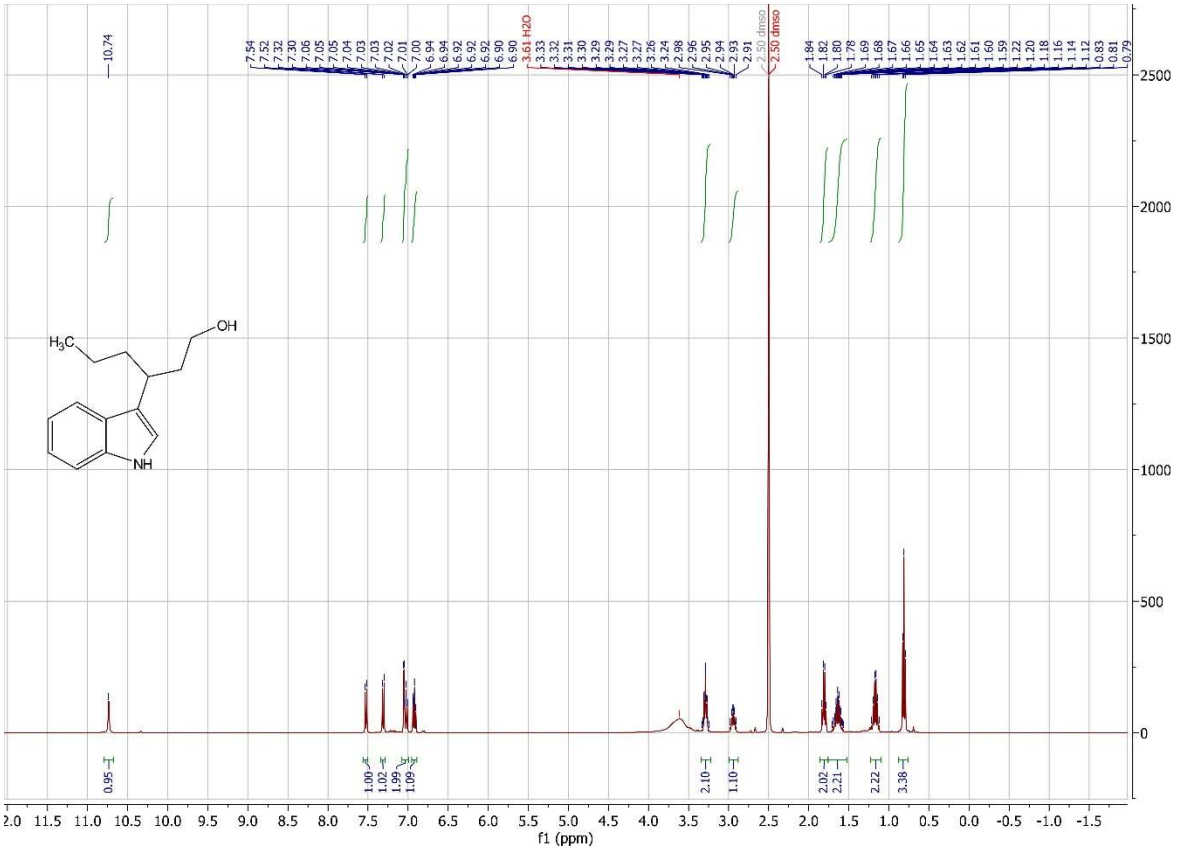


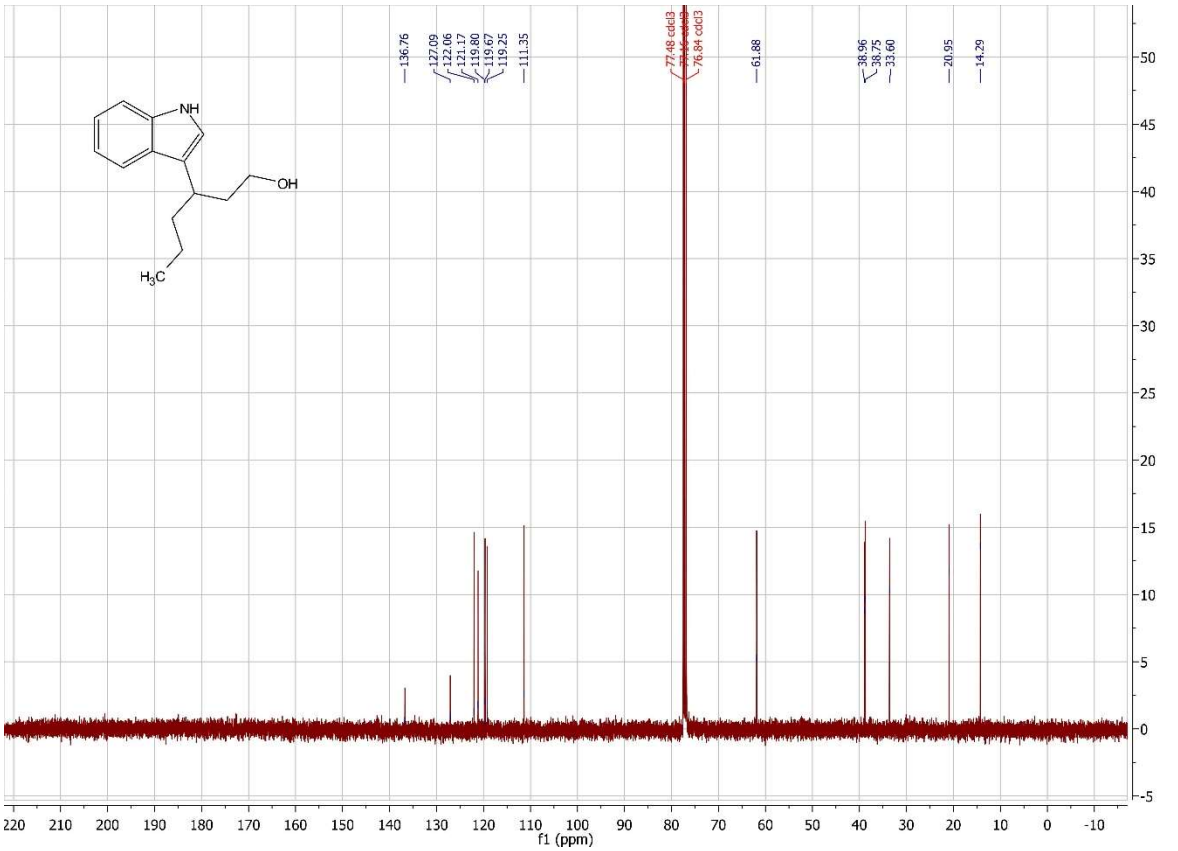
3-(2-methyl-1H-indol-3-yl)-3-phenylpropan-1-ol (3d)



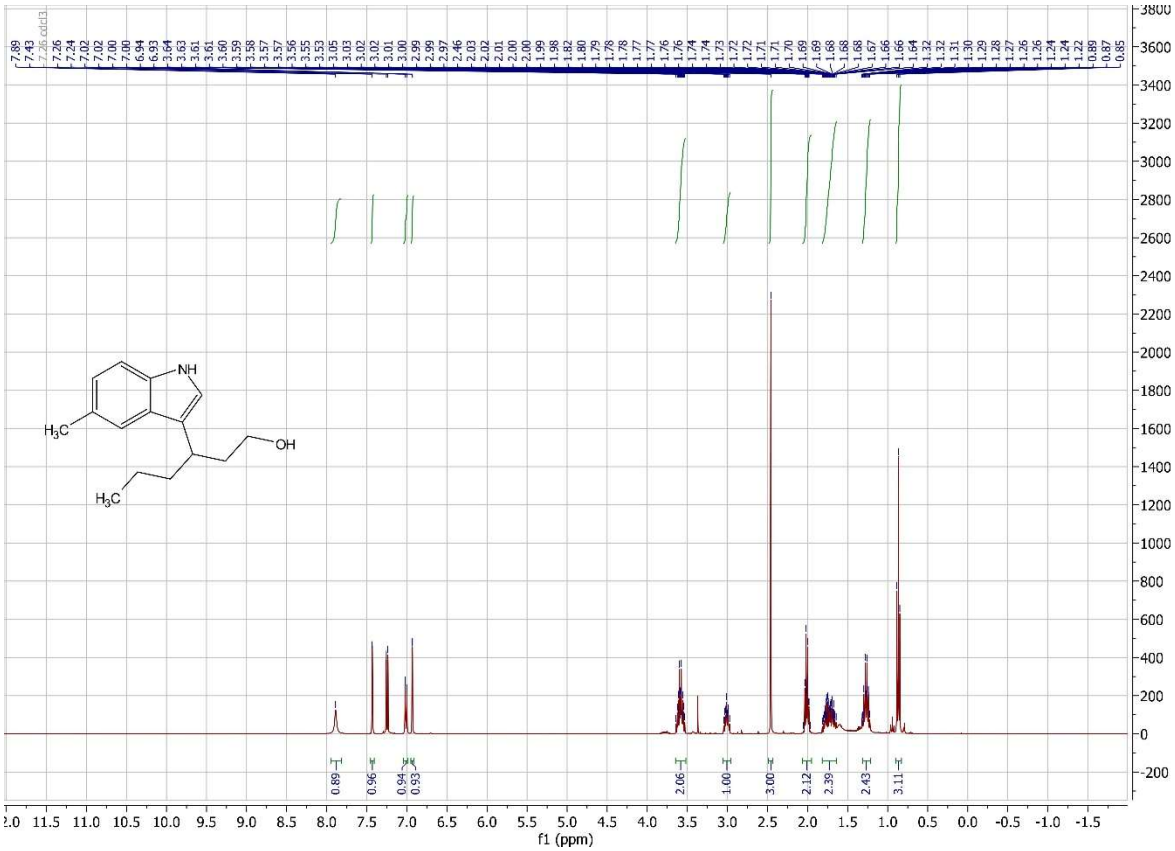


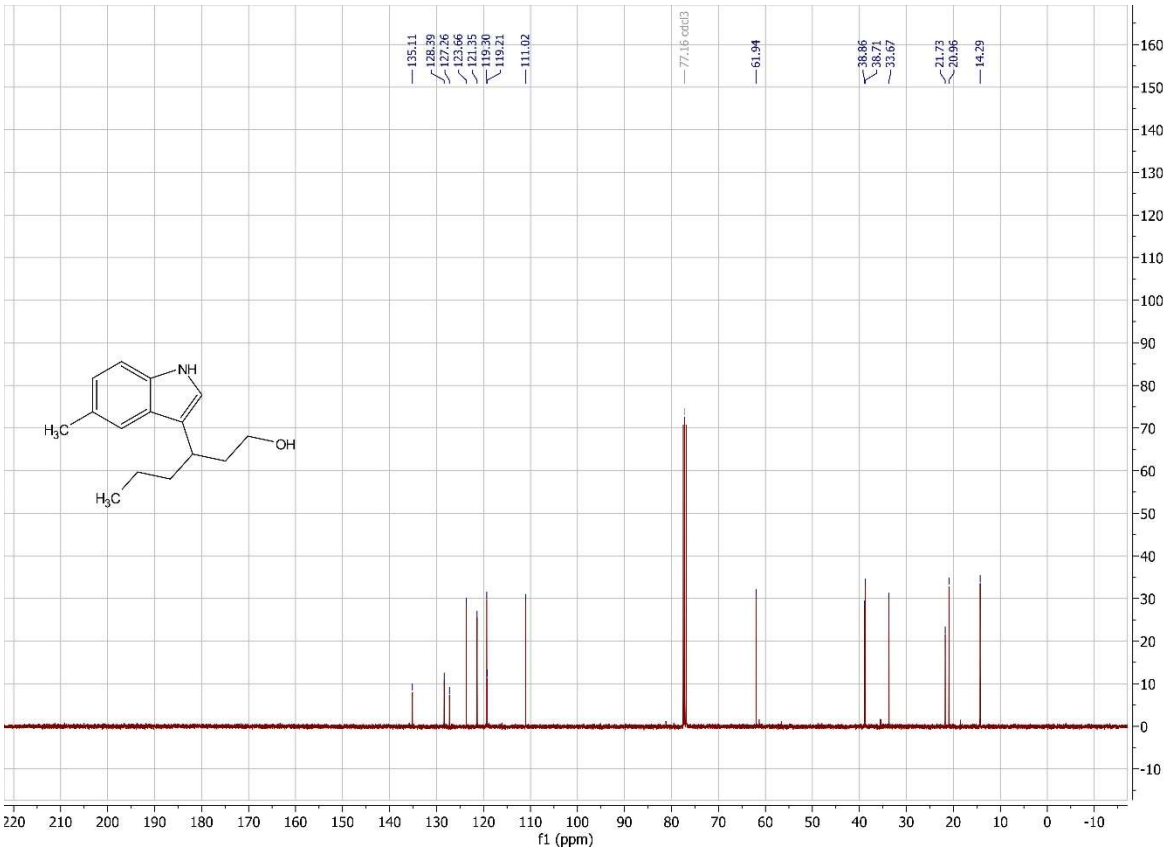
3-(1H-indol-3-yl)hexan-1-ol (3e)



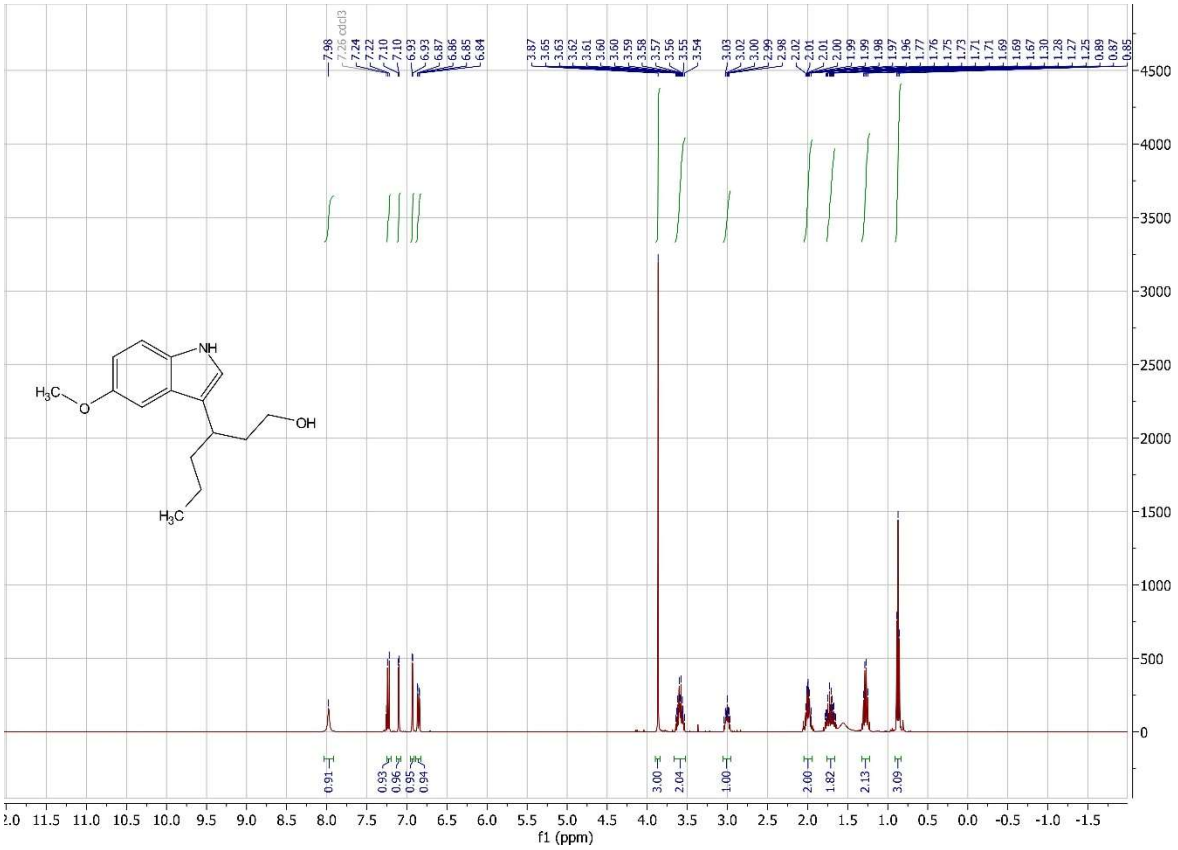


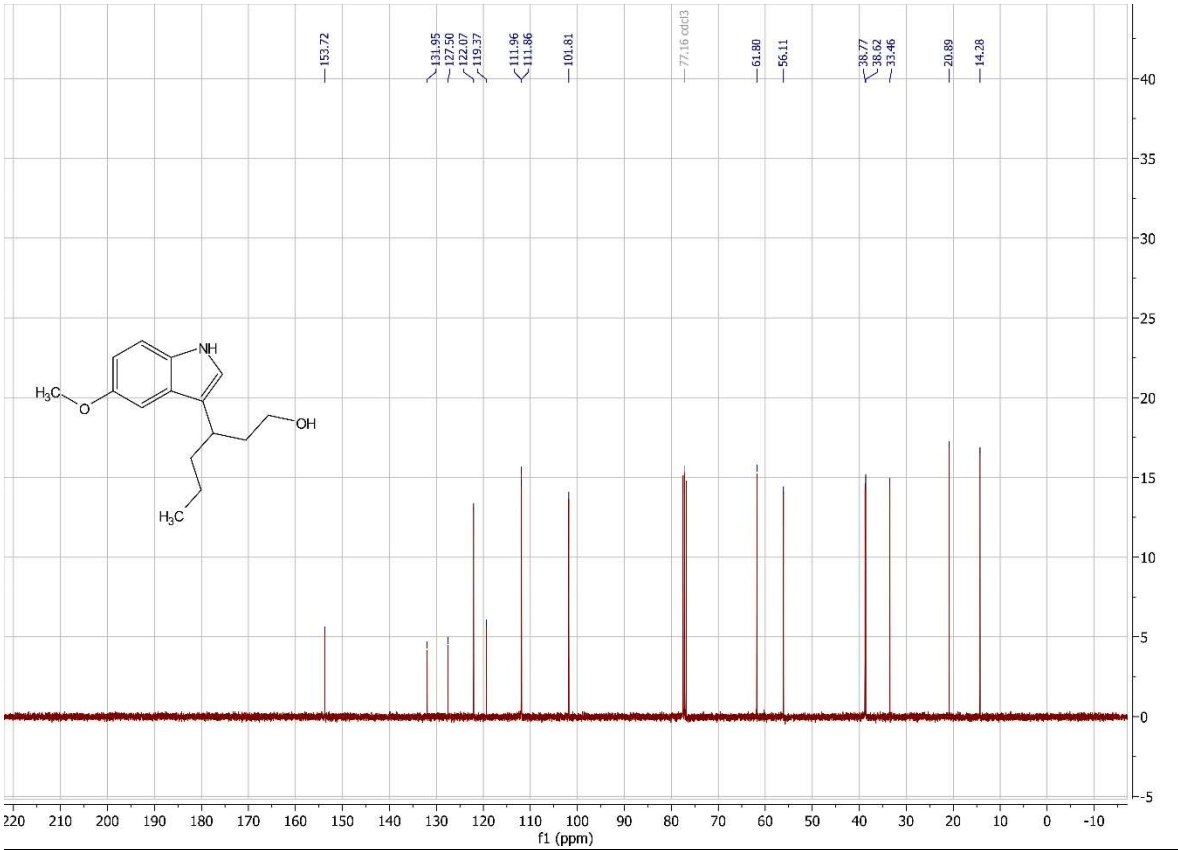
3-(5-methyl-1H-indol-3-yl)hexan-1-ol (3f)



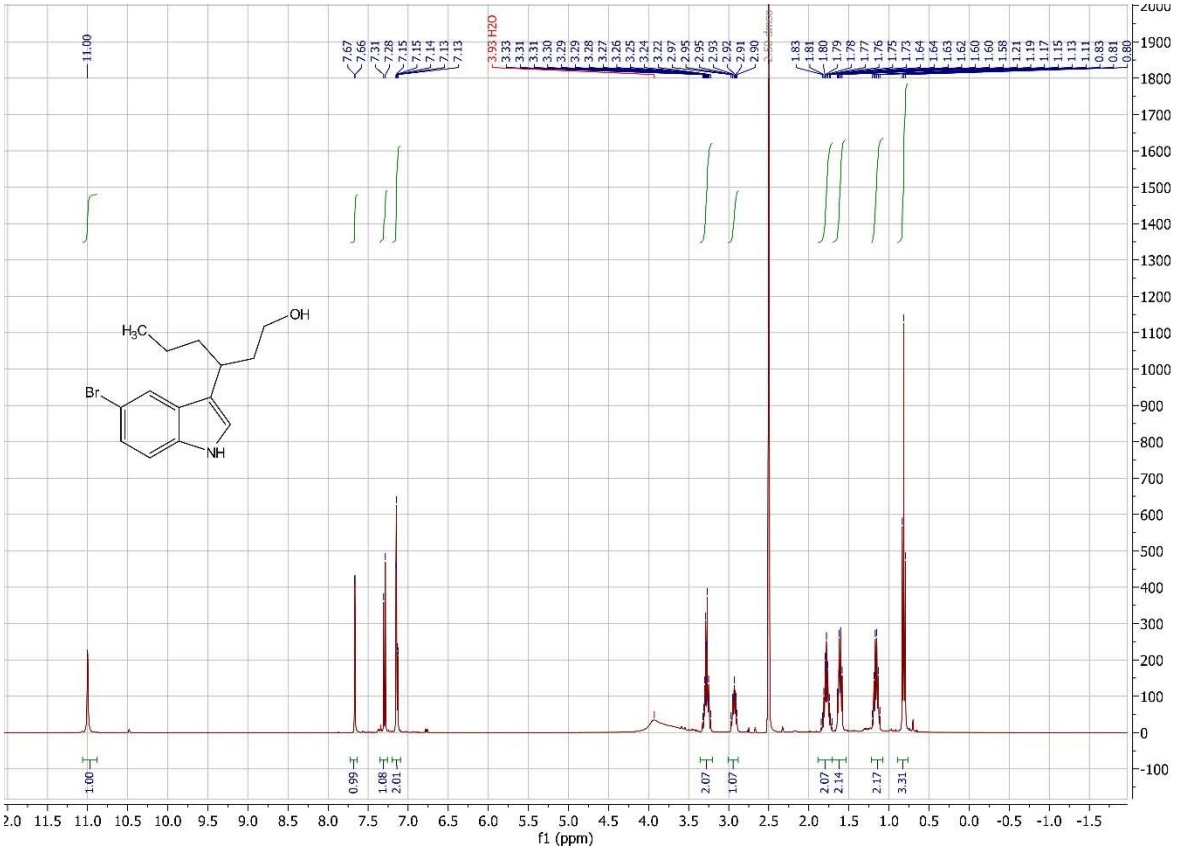


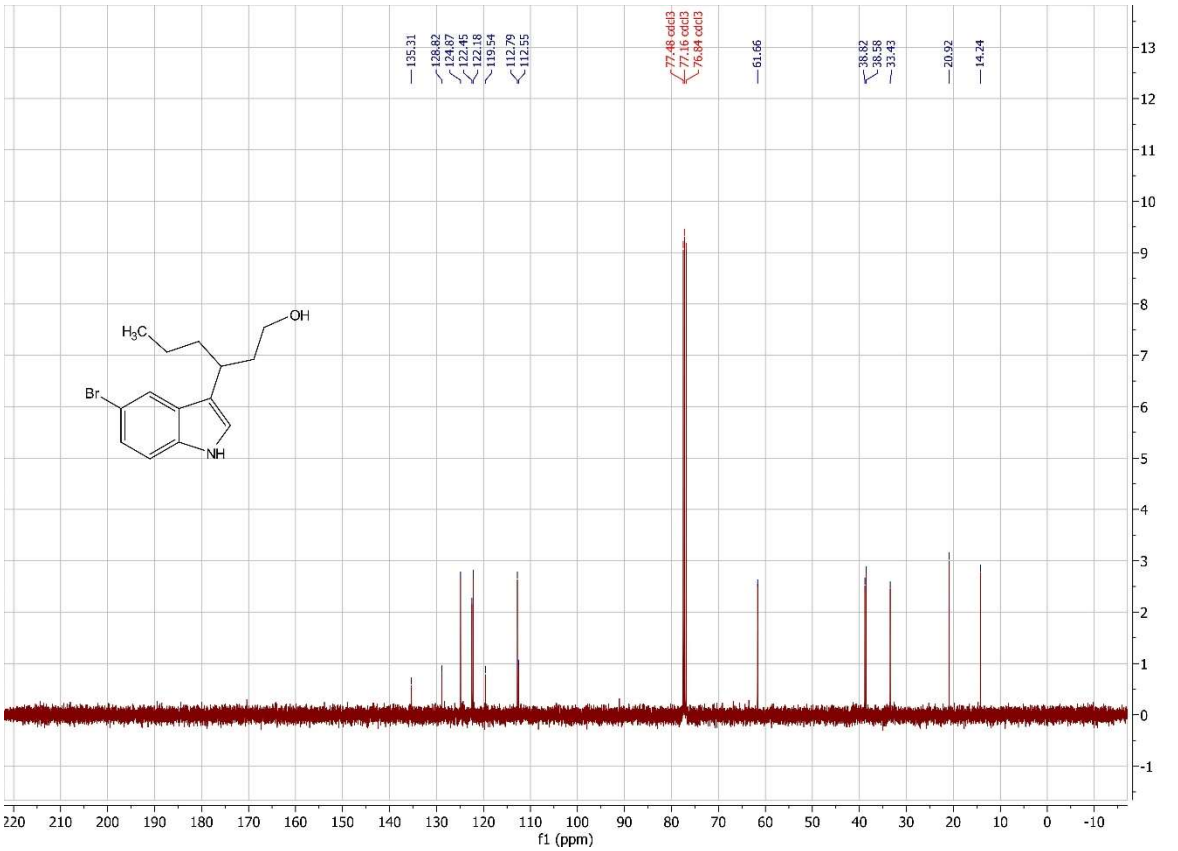
3-(5-methoxy-1H-indol-3-yl)hexan-1-ol (3g)



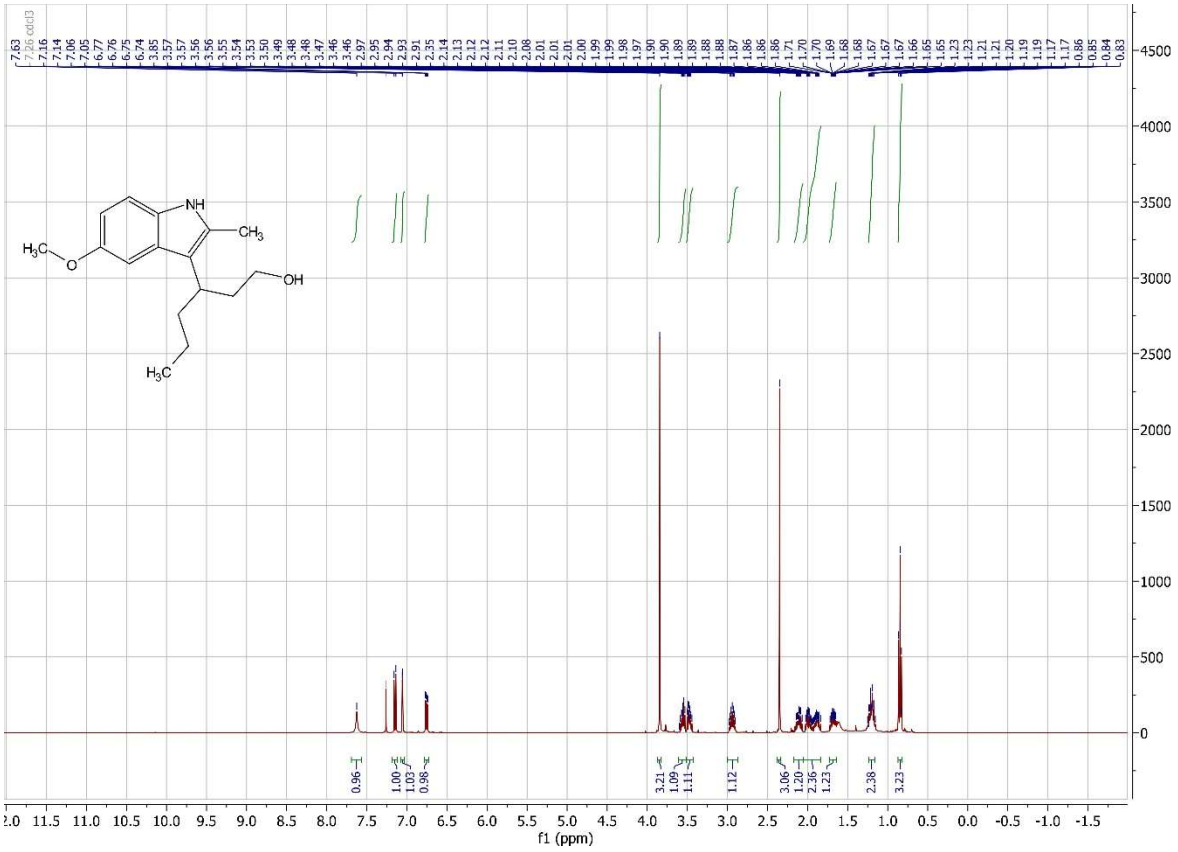


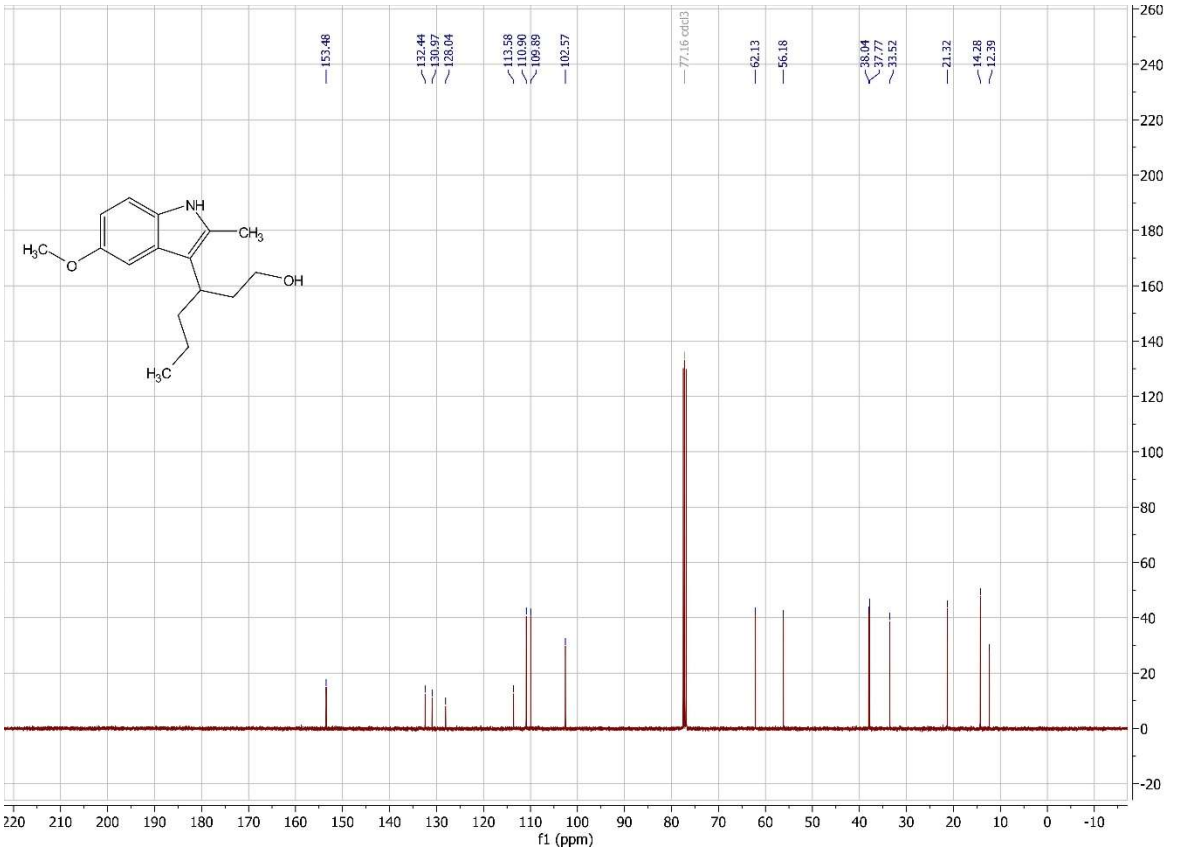
3-(5-bromo-1H-indol-3-yl)hexan-1-ol (3h)



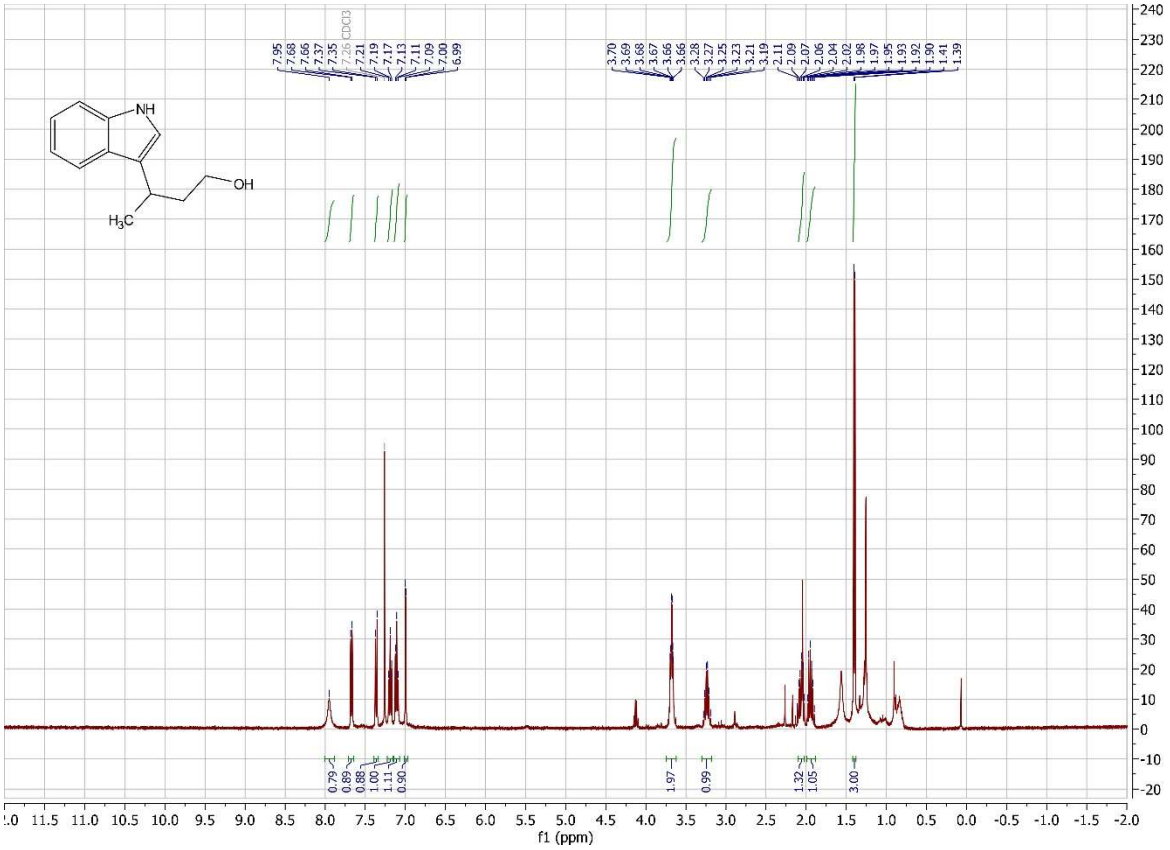


3-(5-methoxy-2-methyl-1H-indol-3-yl)hexan-1-ol (3i)





3-(1H-Indol-3-yl)-butan-1-ol – (3j)



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